ANNUAL REVIEW OF ENTOMOLOGY

EDWARD A. STEINHAUS, *Editor*
University of California

RAY F. SMITH, *Associate Editor*
University of California

VOLUME 4

2334.59 Ann.v.4 c.2

1959

PUBLISHED BY
ANNUAL REVIEWS, INC.
IN CO-OPERATION WITH THE
ENTOMOLOGICAL SOCIETY OF AMERICA

ANNUAL REVIEWS, INC.
PALO ALTO, CALIFORNIA

ANNUAL REVIEWS, INC.
PALO ALTO, CALIFORNIA, U.S.A.

© 1959 BY ANNUAL REVIEWS, INC.
ALL RIGHTS RESERVED

Library of Congress Catalog Card Number: A56-5750

FOREIGN AGENCY

Maruzen Company, Limited, 6
Tori-Nichome Nihonbashi, Tokyo

PRINTED AND BOUND IN THE UNITED STATES OF AMERICA BY
THE GEORGE BANTA COMPANY, INC.

PREFACE

The present volume completes the first five years of life for the *Annual Review of Entomology*. (Although this is only Volume 4, a year of exploration and preparation preceded Volume 1.) During this formative period much has occurred; the *Review* appears to have passed through its "baptism of fire," and, we trust, has taken its place throughout the world as an accepted and stabilized publication in entomology. This accomplishment could not, of course, have been made without the support of many individuals (especially subscribers) and some organizations. In the latter category are the publisher, Annual Reviews, Inc., its Managing Editor, Dr. J. Murray Luck, and the Entomological Society of America.

Although your Editor had been agitating for some type of review publication in entomology since 1945, it was the Society which, through its officers, first gave substance to his hope that such a publication were possible. A general account of the genesis and organizational arrangements pertaining to the *Review* may be found in the *Bulletin of the Entomological Society of America* (Vol. 1, No. 2, 1955). But some of the more intimate details of the *Review's* beginnings are not included in that account. As we glance back, we are again reminded of the great credit that must be extended to men like Charles E. Palm, who, as President of the Society, gave an attentive ear to pleas that the Society take the lead in doing something about the matter. In 1953 he appointed a committee to examine the problem of providing adequate reviews of entomological literature. After a period of arduous work, study, and thorough analysis, the committee recommended that a review publication of the type published by the nonprofit organization Annual Reviews, Inc., would best answer the needs for entomology from an over-all viewpoint. Nor can we forget the interest and support of E. Gorton Linsley, who accompanied the Editor to a crucial meeting with officers of Annual Reviews, Inc., and who, along with others, gave his support to the *Review* when it was being considered by the Society's Governing Board.

From this distance it might appear that it was a logical thing for the Society to give its encouragement and support to the undertaking. It should be remembered, however, that consideration of this matter came at a time when the Society had just completed consolidation, and the demands on the Society's finances were at a peak. Nevertheless, because of the courage, understanding, and farsightedness of the members of this Governing Board and the officers of the Society, the critical decision was made to give the *Review* its moral and financial support according to the terms outlined in the *Bulletin* article to which we have already referred.

As previously agreed, with the completion of the present volume the financial obligations (largely recompensed) of the Society to the *Review* come to an end. Henceforth, Annual Reviews, Inc., will assume this responsi-

bility in full, depending, of course, upon the continued and increasing support (through individual subscriptions) of entomologists everywhere. Especially will the *Review* require the continued support of Society members, who constitute the core of American entomologists. In this connection, it is important to remember that the Society will continue to receive a discount on the retail price of all subscriptions submitted by the Society on behalf of its members. Accordingly, members desiring to benefit the Society while at the same time availing themselves of the *Review* (at the same price) thus have an opportunity to do so. It is appropriate here that all of us directly associated with the *Review* not only register again our thanks and appreciation to the Society for its past enthusiastic and generous support, but also record and acknowledge the fact that without this support the *Review*, once initiated, might never have been able to acquire the use of its wings to soar independently among the best of biological publications.

While it is customary to acknowledge the aid and service of our Editorial Assistant, in the present case this is no mere ritualistic performance. Mrs. Margaret M. Janofsky has not only immensely lightened our editorial load, but has, with outstanding efficiency and effectiveness, handled many of the important details that are associated with the editing and publishing of a first-rate volume.

R.G. C.B.P.
R.L.M. R.F.S.
C.D.M. E.A.S.
C.M.W.

CONTENTS

INSECT BLOOD CELLS, *V. B. Wigglesworth* 1

CULTURE OF INSECT TISSUES, *M. F. Day and T. D. C. Grace* 17

PHEROMONES (ECTOHORMONES) IN INSECTS, *P. Karlson and A. Butenandt* 39

INSECT PIGMENTS, *R. I. T. Cromartie* 59

TAXONOMIC PROBLEMS WITH CLOSELY RELATED SPECIES, *W. J. Brown* 77

ECOLOGY OF CERAMBYCIDAE, *E. G. Linsley* 99

BIOLOGY OF APHIDS, *J. S. Kennedy and H. L. G. Stroyan* 139

THE BIOLOGY OF PARASITIC HYMENOPTERA, *R. L. Doutt* 161

BIOCLIMATIC STUDIES WITH INSECTS, *P. S. Messenger* 183

ETHOLOGICAL STUDIES OF INSECT BEHAVIOR, *G. P. Baerends* . . . 207

EXPERIMENTAL HOST-PARASITE POPULATIONS, *T. Burnett* 235

BIOLOGICAL CONTROL OF WEEDS WITH INSECTS, *C. B. Huffaker* . . . 251

MICROBIAL CONTROL OF INSECT PESTS, *Y. Tanada* 277

ON THE MODE OF ACTION OF INSECTICIDES, *F. P. W. Winteringham and S. E. Lewis* 303

BIOLOGICAL ASSAY OF INSECTICIDE RESIDUES, *S. Nagasawa* 319

DECIDUOUS FRUIT INSECTS AND THEIR CONTROL, *M. M. Barnes* . . . 343

SEED TREATMENT AS A METHOD OF INSECT CONTROL, *W. H. Lange, Jr.* 363

FLEAS AND DISEASE, *W. L. Jellison* 389

INSECTS AND THE EPIDEMIOLOGY OF MALARIA, *P. F. Russell* 415

INDEXES 435

Annual Reviews, Inc., and the Editors of its publications assume no responsibility for the statements expressed by the contributors to this *Review.*

INSECT BLOOD CELLS[1]

By V. B. Wigglesworth
Agricultural Research Council, Unit of Insect Physiology, Cambridge, England

During embryonic development in insects many of the growing organs are overlaid by scattered mesodermal cells (64), some of which give rise to splancnic and somatic muscles, others to fat body, pericardial cells, etc. When embryonic growth is complete, there remains a residue of such cells; some loosely adhere to the surface of the tissues, some freely circulate in the body fluid, some form compact aggregations of fairly constant form and position.

It is these residual mesodermal cells which constitute the "blood cells," or haemocytes, and the question arises whether these cells are still capable, during postembryonic growth, of differentiating to form muscles and other mesodermal tissues. Berlese (14, 15) considered such differentiation to be their main function.

Evidence on this subject is limited. In Hemiptera there are blood cells containing fat droplets [the "adipocytes" of Hollande (25)] which closely resemble the fat body cells. In *Rhodnius* these isolated cells have characters in the nuclei, mitochondria, fat and glycogen content indistinguishable from the typical fat body cells (82). In aquatic Hemiptera, the fat body is said to increase in size throughout life by the incorporation of free adipocytes (54). The fat body arising in place of the flight muscles of queen ants (28), the imaginal fat body of muscids, and the fat body in the elytra of *Haemonia* (Coleoptera) (35) all arise from "mesodermal leucocytes." In the larva of *Oryctes nasicornis* Linnaeus, Lazarencko (39) described rounded blood cells which give rise to new lobes of fat body by collecting in groups or strings and becoming charged with fat droplets.

According to Shatoury & Waddington (67), cells released into the haemolymph from the "lymph glands" of *Drosophila* settle down as "oikocytes" or colonial cells below the imaginal discs and give rise to mesodermal structures in many parts of the body. Similar cells are said to be released from the lymph glands in the latter half of each larval instar and to give rise to the "basement cells" of the alimentary canal, which multiply and form the adult mid-gut (68). (The adult mid-gut in higher Diptera is usually said to arise from the imaginal rings lying at the junction of fore-gut with mid-gut and mid-gut with hind-gut.)

These properties of the haemocytes as "embryonic cells" still capable of differentiating into specialized mesodermal tissues have been inadequately studied. It is not easy to draw a distinction between the spindle-shaped myoblasts below the imaginal discs and the very similar haemocytes (37, 48). We shall be concerned in this review with the little that is known about the function of the haemocytes as such. We shall deal mainly with work pub-

[1] The survey of the literature pertaining to this review was completed in March, 1958.

lished during the past eight or ten years, since the textbook reviews of Munson (45) and the present writer (76) were prepared; but some reference to earlier work will be necessary. The papers of Ermin (19) and Rooseboom (60) provide a useful guide to the earlier literature.

CIRCULATING AND FIXED HAEMOCYTES

There are many variations among different insects in the extent to which the haemocytes are freely circulating. In fixed specimens of *Periplaneta americana* (Linnaeus), *Tenebrio molitor* (Linnaeus), *Galleria mellonella* (Linnaeus), *Heterocampa manteo* Doubleday, *Pieris* spp., and *Apis mellifera* Linnaeus, there are usually abundant free haemocytes in both the heart lumen and in the haemocoele; and numerous free haemocytes may be seen to enter the heart in living specimens of *Blattella germanica* (Linnaeus) (30). But in many insects—in *Rhodnius* (82) or the larva of *Phormia* (30) for example—they appear not to enter the heart. In such insects the blood cells cannot all be free and unattached in the body cavity, or they would be carried to the regions of the ostia of the heart. They must be largely adherent to the tissues while the haemolymph is circulated past them. That is so in *Culex pipiens* Linnaeus, *Aedes aegypti* (Linnaeus), *Anopheles quadrimaculatus* (Say), *Chironomus* spp., *Pediculus humanus* Linnaeus (30), in the larva of *Musca* (6), and in many other insects. In the wings of *Drosophila* there is a regular circulation of blood, aided by at least five accessory pulsatile organs, but only during the first hour of adult life may moving haemocytes be observed (53).

Haemopoietic organs.—There are all intermediate stages between freely circulating blood cells, cells loosely adherent to the tissues or forming transient aggregations, and collections of cells which form distinct organs of more or less constant position and form. Such permanent aggregations are often regarded as blood-forming or haemopoietic organs or *Bildungsherde*. They may occur in many different parts of the body.

In the larva of *Oryctes* they overlie the esophagus, close to the corpora allata (39). In the head of *Corixa punctata* Illig there is a tubular organ with membranous walls bearing scattered blood cells, which is perhaps a haemopoietic organ (13). Haemopoietic organs enclosed in delicate membranes occur in the outer layer of the fat body in each body segment of sawfly larvae (Tenthredinidae). They disappear at the beginning of the pupal stage (40).

Similar organs have been described by Arvy (4) in the thorax of first instar larvae of various Lepidoptera. Here they form four masses placed a little behind the prothoracic spiracle. They possess a capsule and an intercellular reticulum; they are clearly organs and not temporary accumulations of haemocytes. Mitoses are numerous in them; they increase in size at successive larval stages, and all steps in the differentiation of the haemocytes may be followed. There are gaps in the capsule through which these cells escape into the circulating blood. The liberation of haemocytes reaches its

maximum in the pupa; in the adult the organs have completely disappeared.

The transition from free haemocytes to compact blood tissue is well seen in chironomid larvae (36). In the larva of *Chironomus plumosus* (Linnaeus) circulating cells are entirely wanting, but haemopoietic tissue occurs in the form of two longitudinal columns in the eleventh and twelfth segments near the anal appendages (5, 36). In *Calliphora* no haemocytes appear until the end of the first larval stage. They are present in the second and third stages, being derived from haemopoietic masses close to the posterior spiracles. *Musca* and *Phaonia* lack free haemocytes throughout larval life. They have a well-developed haemopoietic organ lying against the dorso-lateral epidermis near the posterior spiracles, and at the time of puparium formation this organ dissociates to produce free haemocytes (5). In the white puparium of *Musca* the organ has broken down completely and the haemocytes are applied to the muscles; by the end of 24 hr. no trace of the organ remains (6).

HAEMOCYTE COUNT

There is abundant evidence that a large and varying proportion of the blood cells are normally sedentary. Not only are they loosely adherent to the body wall and the surface of the organs, but they insinuate themselves in great numbers between the fibres of developing muscles (78) and probably into other organs. Ovarian tissue of *Bombyx mori* (Linnaeus) larvae in tissue culture gives rise to dividing cells which superficially resemble vertebrate fibroblasts in open growth (83). It is not improbable that these represent haemocytes formerly adherent to the tissues.

It follows, therefore, that counts of haemocytes in samples of drawn blood must include only the free cells and those most loosely attached and that the figures are likely to be variable. Jones & Tauber (33), using heat-fixed *Tenebrio* larvae (55°C. for 2 min.), obtained an average count of 48,000 cells per mm.^{3} with a standard deviation of 17,000. In unfixed larvae the numbers obtained were significantly less: approximately 20,000 cells per mm.^{3} were held back (31).

But while the actual counts of blood cells have little significance, the changes in the numbers at different times in the life cycle are important. Recent results have confirmed those of the past in showing that haemocytes are most numerous during the phases of active growth. In *Ephestia* the numbers decline when the pupa becomes adult (3). In *Leptinotarsa decemlineata* (Say), there is a progressive increase during larval life: after each moult the total numbers are reduced and then increase again as growth is renewed (7). The same cycle occurs in *Locusta migratoria* (Linnaeus) (73).

In the larva of *Sarcophaga*, as studied by Jones (32), the two most abundant types of blood cells, the "plasmatocytes" and the "granular haemocytes," reach their peak in the prepupal stage. As the larva develops from the "crop-full" stage to the prepupa, the number of plasmatocytes in unfixed

blood doubles, and the number of granular haemocytes increases sevenfold, the total count rising from less than 8000/mm.^{3} to about 34,000/mm.^{3} without any change in blood volume. On the other hand, the number of "spherule cells" is greatest in the crawling crop-full larva a day or so before pupation. When pupation begins, the number of haemocytes in the unfixed blood falls abruptly, and within 6 hr., when the puparium is brown, about two thirds of the haemocytes are no longer in circulation. Finally, both granular haemocytes and spherule cells disappear, and, consequently, they may be considered as larval cells. Plasmatocytes persist into the adult stage. By comparison with other Diptera (6), it is probable that the great increase in the haemocyte count before pupation is caused by the sudden release of cells from haemopoietic organs. The sudden fall at pupation presumably results from their adhesion to tissue surfaces (32).

TYPES OF HAEMOCYTES

The naming and classification of the different kinds of haemocytes are rendered difficult by the varying techniques for staining that have been employed (26), by the great diversity of insects, and by the extreme variability in the histological characters of the cells within a single insect. This last factor enabled Yeager (85) in his well-known work on the larva of *Prodenia eridania* (Cramer) to recognize 10 classes containing 32 types. The classes were as follows: (*a*) proleucocytoids, (*b*) small chromophil cells derived from (*a*), (*c*) oenocytoids, (*d*) plasmatocytes with finely vacuolated cytoplasm, (*e*) podocytes—thin, flat cells with pseudopodia-like extensions, (*f*) vermiform cells, (*g*) cystocytes with rounded eosinophil inclusions, (*h*) spheroidocytes with colourless vacuoles, (*i*) eruptive, unstable cells, (*j*) degenerative cells.

It may be useful, without recording the detailed descriptions, to summarize some of the classifications published in recent years. In the adult of *Forficula auricularia* Linnaeus, Arvy & Lhoste (8, 40a) find four types: (*a*) leucoblasts (mother cells), (*b*) small, rounded basophil leucocytes, (*c*) leucocytes with "dusty" cytoplasm, and (*d*) leucocytes with crystalloids. In the larva, in addition, megacytes are present (9). In Orthoptera, Ögel (49) always found four groups, (*a*) mother cells, (*b*) proleucocytes, (*c*) phagocytes, and (*d*) granular leucocytes. In *Rhodnius* the present writer (78) recognizes only (*a*) proleucocytes, (*b*) phagocytic amoebocytes of highly varied form (probably embracing plasmatocytes, podocytes and spheroidocytes) and (*c*) oenocytoids. In addition to these, there are also adipocytes (free fat body cells) and two infrequent forms, probably derived from (*b*): large granular and nongranular cells. In *L. decemlineata* (7) there are (*a*) leucoblasts, (*b*) leucocytes with basophil cytoplasm, (*c*) leucocytes with "powdery" cytoplasm and (*d*) leucocytes with fuchsinophil inclusions. In the larva of *Tenebrio*, Jones (31), using the phase contrast microscope, describes (*a*) plasmatocytes, 56 per cent, (*b*) cystocytes, 43 per cent, and (*c*) oenocytoids, 0.6 per cent. In *Anagasta kühniella* (Zeller),

Arnold (3) finds four classes: (*a*) prohaemocytes, (*b*) plasmatocytes (phagocytes), (*c*) spheroidocytes, and (*d*) oenocytoids. In the honey bee, Morgenthaler (43) recognizes only three cell types: proleucocytes, leucocytes, and pycnonucleocytes, the latter being large cells with an eccentric, darkly staining nucleus. In the larva of *Drosophila willistoni* (Metz), Rizki (58) finds (*a*) plasmatocytes making up 70 to 90 per cent of the total count, except at the end of larval life; (*b*) podocytes, which become the main form at the time of pupation; (*c*) spheroidocytes; (*d*) oenocytoids; (*e*) nematocytes; (*f*) crystalloid cells, limited to the first and second instars. In *Drosophilia melanogaster* Meigen the blood cell population is somewhat different: crystalloid cells occur all through larval life (59). Shatoury (66), on the other hand, claims that blood cells do not exist in the larva of *D. melanogaster* but that the "lymph glands" liberate three types of cells, termed "hexagons," "spheroids," and "platelets," which settle as oikocytes below the developing imaginal discs (p. 1). Dennell (18) recognizes four types in *Sarcophaga falculata* Pandelle: proleucocytes, phagocytes, granular leucocytes and oenocytoids. The last three of these clearly correspond with the three major groups found by Jones (32) in *Sarcophaga bullata* Parker, namely, (*a*) plasmatocytes, (*b*) granular haemocytes, and (*c*) spherule cells. In *Calliphora*, Åkesson (1) recognizes seven types of blood cells, but among the other rather less well-defined types the proleucocytes and the three forms listed by Jones in *Sarcophaga* are perhaps the most clearly characterized.

In the present state of knowledge it is of little use to attempt any precise homology between these varied types. Only a few simple generalizations can be made:

(*a*) The small, rounded proleucocytes with scanty, darkly staining cytoplasm, which are often seen undergoing mitosis, are certainly stem cells which probably give rise to all the other types.

(*b*) The most plentiful cells are usually phagocytic and these cells can assume the most diverse forms. When floating freely in the blood, they are smoothly rounded or oval; when examined in the fresh state or in osmium-fixed material, the sedentary cells always have abundant filiform prolongations. These cells may become vacuolated or drawn out into long spindle-shaped forms, or may spread out extremely flat on the basement membranes (77, 78).

(*c*) There are a number of nonphagocytic cell types which seem to be characteristic of particular groups of insects, such as the well-defined spherule cells of *Sarcophaga* (32), the "wax cells," "carmine cells," "eosinophil cells," and cells with phenolic inclusions in the coccid *Pulvinaria mesembryanthemi* (Vallot) (55) or the "crystalloid cells" of first and second stage *Drosophila* larvae (58).

(*d*) The oenocytoids form a well-characterized type of cell in those groups in which it occurs, the Hemiptera, Coleoptera, Lepidoptera, and Diptera.

We shall return to the description of some of these types when discussing their functions.

The proportions of the different cell types often change during the life cycle. There is a tendency for the proleucocytes to be relatively more plentiful in the young stages, while the more differentiated forms predominate later—for example, in *Forficula* (8) and *Leptinotarsa* (7). In the full-grown larva of *Calliphora* most cells are proleucocytes; in the young prepupa granular cells are most plentiful, and in the older prepupa and pupa, spherule cells predominate (1). In the early larva of *Ephestia* the majority of blood cells are plasmatocytes, while at the end of larval life most are spheroidocytes loaded with fat droplets. All forms decline rapidly at pupation and few persist in the adult (3).

Finally, it may be recalled that Liebman (41), after studying insects along with other invertebrates, recognized only two main groups of blood elements originating from a common stem cell: (*a*) phagocytes (lymphoidocytes) and (*b*) nonphagocytic cells containing granular or spherical inclusions (trephocytes), the oenocytoids being perhaps trephocytes which have discharged their inclusions. Such a simplification would certainly be welcome; but at the present time the prime need is to discover the functions of particular cells; classification can follow.

FUNCTIONS OF BLOOD CELLS

Phagocytosis and Immunity

In considering the functions of the haemocytes it will be well, so far as possible, to concentrate on those functions that have been clearly established by observation and experiment. The foremost of these is phagocytosis. The classic theory of inflammation dates from the observations of Metchnikoff on the blood cells of *Daphnia*. The majority of insect blood cells are phagocytic and ingest microorganisms, tissue debris, and foreign matter of all kinds. For review and bibliography of the earlier literature see Ermin (19).

The blood cells become most numerous during moulting and metamorphosis when many tissues are breaking down; and in some forms—for example, *Calliphora* as studied in the classic work of Pérez (52)—abundant fragments of histolysing tissues are ingested by the blood cells. But there is great variation in this respect; in many insects with extensive histolysis during metamorphosis, phagocytosis seems to play no part at all (76, p. 51). A recent example may be quoted. In the larva of *Rhodnius* the ventral intersegmental muscles of the abdomen are functional only during the act of moulting; the fibres then break down completely and only the muscle sheath and nuclei persist. This breakdown is a pure autolysis: the haemocytes take no part at all and do not even collect in the neighbourhood of the dissolving muscles (79).

It is possible, of course, that much of the normal phagocytic activity of

the blood cells may be imperceptible. The cytoplasmic processes of the podocytes in the *Drosophila* larva are so fine that they are visible only by phase contrast. These delicate filaments are said to beat rapidly, and minute particles in the haemolymph adhere to them (58). There is no doubt that the capacity of the blood for phagocytosis is increased during the moulting periods. For example, the stomach of the bedbug *Cimex* may, after a meal of blood, sometimes rupture so that red corpuscles are set free into the body cavity. Here they may persist unaltered for several weeks (74), but if the insect moults they disappear completely (34).

Apart from the phagocytosis of tissue debris, the blood cells unquestionably play a part in protection and immunity from infective organisms. The classic work in this field is reviewed by Huff (27) and Toumanoff (71). Most of this work deals with microorganisms artificially introduced into the insect. But Tauber (69) showed that when *Blatta orientalis* Linnaeus is infected naturally with *Staphylcoccus albus* the proportion of mitotically dividing blood cells rises from the normal 0.5 per cent to 3.1 per cent; the increase may continue until the blood becomes milky (70).

Protection from Metazoan Parasites

In the past there has been some disagreement over the extent to which the phagocytic blood cells are concerned in the varying degrees of immunity shown by insects toward different entomophagous parasites. Recent careful experimental work has confirmed that the phagocytes are important in the immunity reaction. Thus the inhibition of the development of parasite embryos of *Mesoleius tenthredinidis* Morley in the larch sawfly (*Pristiphora erichsonii* Hartig) seemed to be related to the deposition of phagocytic capsules around them. It was found by Muldrew (44) that such capsules were formed in host larvae from Manitoba and Saskatchewan, but not in larvae from British Columbia. In this case the encapsulated embryos might remain viable for several months.

Two types of protective reaction against the parasite *Diplazon fissorius* (Gravenhorst) were observed in syrphid larvae by Schneider (65). The commonest type occurs in *Syrphus ribesii* (Linnaeus) where a thick gelatinous capsule with nuclei is formed which arrests embryonic development at an early stage. In *Epistrophe balteata* (Degeer) a thin, tough, brown capsule without nuclei is formed. This sheath does not arrest embryonic development, but it prevents mechanically the escape of the young larva.

These protective responses are much more evident in the larger host larvae. Schneider (65) obtained evidence that the serosa of *D. fissorius* produces toxins which seem to arrest the protective response of the host, especially the conversion of haemocytes into capsule substance. This action will naturally be more effective in the smaller hosts; hence the protective reaction in these is readily arrested. Thus *Diplazon laetatorius* (Fabricius) meets a very active protective reaction in its normal host *E. balteata;* but

because the egg is usually deposited in very young hosts, it is ordinarily able to survive. *D. fissorius* is a normal parasite of *Epistrophe bifasciata* (Fabricius); during diapause the young larva is gradually encapsulated, but it is able to free itself mechanically.

In an extensive series of experiments, Salt has implanted the eggs of the ichneumon fly *Nemeritis canescens* (Gravenhorst) into a wide variety of insects and observed the reactions in great detail. When the eggs were introduced into eight different Lepidoptera, the results were as follows (61). In two species the reactions were feeble or absent, and the parasite continued its development. In three species the eggs were encapsulated by haemocytes and prevented from hatching. In three other species the egg hatched, but the host reacted to the parasite larva to prevent its development—in two species by encapsulation, in one by blocking its alimentary canal with melanin.

Eggs injected into the adult stick insect, *Carausius morosus* Brunner (62), provoked the same reactions: investment by haemocytes and melanization. The melanization was progressive and finally resulted in encasing the parasite in a brittle black sheath. The haemocyte reaction was not prevented by the injection of Chinese ink, for blood cells filled with carbon particles nevertheless invested the parasite. Melanin formation was inhibited by the simultaneous injection of phenyl thiourea. Both reactions were elicited by living and by dead parasites, but in the presence of clean glass rods the haemocyte reaction occurred without melanization. Clearly some material, either enzyme or substrate, is furnished by the parasite and reacts with the disintegrating blood cells to produce melanin. The tough, brown capsule formed in some *Epistrophe* larvae would appear to be composed of sclerotin, the formation of which is closely related to melanin (80).

Most of the eggs in *Carausius* failed to hatch, although the fully developed larvae were alive within them. Since they hatched and survived in hanging drops of *Carausius* blood, the death of the parasite is clearly caused by the haemocyte and melanin reactions.

Larvae and pupae of *Diataraxia oleraceae* (Linnaeus) (Lepidoptera) (63) rapidly encapsulated injected parasites, but the adults gave no visible reaction and the parasites continued to grow until the death of the host. This is probably correlated with the reduced number of haemocytes that occurs in many adult Holometabola. On the other hand, in larvae, pupae, and adults of *T. molitor* the eggs hatched, but within 24 hr. haemocytes enveloped the parasite larvae and melanin was deposited around the mouth and anus. Salt suggests that the interference with feeding may be a further cause of death (63).

Salt is inclined to attribute the death of the larvae in most of these experiments to physical restraint by the haemocytes. But it is possible that in some cases the failure to hatch, the arrest of growth, and the eventual death may be brought about simply by interference with the supply of oxygen.

Blood Coagulation

Studying the coagulation of insect blood, Beard (12) found a clear distinction between the larva of *Popillia,* in which the haemolymph coagulates by gelation of the plasma and the haemocytes do not agglutinate, and the larva of *Galleria,* in which coagulation is dependent on agglutination of the cells.

The subject has been much clarified by the researches of Grégoire & Florkin (23). Taking *Carausius* as a typical example, the haemolymph as seen with the phase-contrast microscope contains four types of cells: (*a*) small, round hyaline haemocytes, (*b*) large granular haemocytes, (*c*) large hyaline forms with a small nucleus, and (*d*) coarsely granular haemocytes. The cells of type *c,* the large hyaline cells, Grégoire & Florkin term "coagulocytes." Like the "explosive corpuscles" described by Hardy in Crustacea toward the end of the last century, insect coagulocytes are characterized by an increased sensitiveness to contact with nongreasy foreign matter. On contact with the surface of the slide or other objects, they show a whirling and bubbling of the cytoplasm. Substance is ejected from the cell and leads to the appearance of a granular cloud which spreads out over a diameter several times that of the cell and then stops. This process is followed later by coagulation of the plasma surrounding these islands. These observations are confirmed by Czihak (17), and it is worth noting that in the blood of *Carausius* and *Forficula,* Rooseboom (60) observed nuclei in fragments of hyaline cytoplasm in about the same percentage as the coagulocytes of Grégoire.

More recently Grégoire (20, 21, 22) has studied blood coagulation in more than 400 species of insects. The observed changes fall into four categories: (*a*) The plasmatic reaction develops around most of the hyaline haemocytes in the form of islets of coagulation which extend progressively. (*b*) A network of pseudopodial strands is produced by the hyaline haemocytes; plasmatic reaction in the form of a mist is confined to the system of strands. (*c*) A combination of (*a*) and (*b*). (*d*) Hyaline haemocytes are inert and show no apparent alteration. The plasmatic reaction is absent. In most of the Hemiptera studied, including *Rhodnius* and other Reduviids (in which clotting does not occur), the reaction is of type *d.*

In almost all species the hyaline haemocytes are easily recognized by phase contrast, but it is not possible to identify them with certainty in fixed preparations or to ascribe them to any of the usual types described in fixed preparations (p. 4). In Hemiptera they may correspond to the oenocytoids —but in most forms the hyaline cells are phagocytic, which the oenocytoids are not.

In the larva of *Sarcophaga,* Jones (32) found no tendency for any of the cells to discharge their contents into the plasma when the blood is withdrawn. None of the haemocytes are associated with islands of coagulation, but the haemolymph of large, crop-full larvae begins to gel in about 15 min. and is completely gelled in about 40 min. Similarly, in the larva of *Calli-*

phora, Åkesson (1) found that clotting could take place in the plasma after removal of the cells—though, as Hinton (24) points out, a factor may already have been liberated from the coagulocytes. Heat treatment (60°C.) is effective in preventing coagulation in *Popillia* and *Galleria* larvae (12) only if it is applied to the intact insect; it may well be acting upon the coagulocytes. A long series of well-known inhibitors of coagulation have been tested on insect blood (21) and a good proportion of these, including calcium precipitants, are said to be effective. In general, when the substance tested prevented the characteristic morphological changes in the hyaline haemocytes, coagulation did not occur.

Connective Tissue Formation

The presence of thin strands and sheets of connecting tissue in insects is well recognized (10, 11, 39, 47, 57, 72), but little attention has been given to the method of their formation. The films and lamellae without fixed cells which constitute the basement membranes and the membranes around the fat body, etc., as described by Lazarenko (39), contain no elements corresponding with the fibroblasts of vertebrates. Such connective tissue formations can be provoked by implanting tubes of celloidin. The sole source of the ground substance of which they are composed is the spindle cells of the haemolymph, which accumulate around the foreign body, phagocytose detritus, and then break down to yield a capsule of ground substance, the nuclei disappearing. It was suggested by Lazarenko (39) that normal connective tissue is probably formed in the same way. He regarded the cells concerned, not as blood cells, but as special mesenchyme cells from the tissue spaces. The same interpretation was put by Lartschenko (38) on the encapsulation of hymenopterous parasites in caterpillars.

Palm (51) described a tissue element in Odonata (*Aeschna*) and Diptera (*Eristalis*)—which he was "inclined to refer to as a form of connective tissue"—produced by fixed haemocytes resembling the histiocytes of vertebrates. These cells merge in the one direction with connective tissue cells forming part of a membranous tissue and in the other with the ordinary haemocytes. All these cells take up injected trypan blue.

A reinvestigation of connective tissue formation in *Rhodnius* has confirmed and extended these observations (78). The most abundant type of haemocyte, the phagocytic "amoebocyte" is characterized by containing rounded, oval, or rodlike glassy inclusions. These stain supravitally with gentian violet, taking on a slate-blue tint; but their most important property is that of giving a strongly positive periodic acid-Schiff (PAS) reaction unaffected by treatment with saliva. These inclusions are believed to consist of some neutral mucopolysaccharide. They stain green with Masson's trichrome, blue-black with the chrome-haematoxylin of Gomori and deep red with fuchsin-paraldehyde. All these staining properties they have in common with the basement membranes and connective tissues, and it is claimed that they are the precursor of the connective tissue substance. Ohuye

& Horikawa (50) have reported the presence of a neutral mucopolysaccharide in the "basophilic granulocytes" of *Xylotrupes*.

Apart from the similarity in staining and histochemical reactions, the evidence is as follows. During each moulting stage the amoebocytes multiply extensively. Shortly before the new cuticle is formed, they migrate in large numbers to the epidermis and settle down below the basement membrane. By this time they are very rich in PAS-positive inclusions. They spread out over the basement membrane and may be seen to discharge their inclusions, which merge into the substance of the membrane. The same process may be observed in the formation of the membranes and filaments of connective tissue around the fat body. And finally the amoebocytes insinuate themselves in large numbers between the fibres of developing muscles and give rise to the PAS-positive muscle sheaths. They may contribute also to the sheaths around the nerves and ganglia. Johannson (*29*) produces evidence that the haemocytes are intimately associated with the perilemma in the ganglia of *Oncopeltus*.

It has recently been discovered (82) that the inclusions in the amoebocytes will bind osmium and can thus be very clearly demonstrated by the osmium-gallate method of staining (81). This has made possible the confirmation of the observations described above. Whether the inclusions contain unsaturated lipides in combined form or some other osmium-binding group (perhaps sulphydryl groups) has not been determined.

Role of the Blood Cells in Intermediary Metabolism

Under this heading are to be found perhaps the most important functions of the haemocytes. But the nature of these functions is still largely unknown, and in this section we shall be concerned mainly with listing possibilities.

Protein synthesis.—Cuénot (16) suggested that the haemocytes had three main functions: phagocytosis and the formation of the haemostatic plug, transport of reserves, and the production of the free proteins of the blood. There is no doubt that the protein synthesis necessary for the growth of the haemocytes themselves takes place in these cells. Yeager (84) obtained histological evidence of the transfer of nucleolar material of the blood cells of *Prodenia* to the cytoplasm. This might be regarded as some slight evidence of ribonucleic-acid transfer associated with protein synthesis. But nucleic-acid and protein synthesis is quantitatively far more important in the fat body and the latter might equally well be the source of the blood proteins, as, indeed, might the pericardial or other cells.

Transfer of nutrients.—Cuénot (1891) and Berlese (14, 15) considered this one of the main functions of the haemocytes. Glycogen is plentiful in the blood cells of well-fed larvae of *Prodenia* (86), especially during the prepupal period when glycogen deposits are visible in practically all tissues; it falls rapidly to a low level early in metamorphosis. In *Rhodnius*, glycogen appears in relatively small amounts in the epidermal cells, but,

apart from the adipocytes, which seem to be identical with fat body cells, glycogen is not to be seen in any of the haemocytes (78). The blood cells of *Prodenia* on a normal diet come to contain fat inclusions in the prepupal stage and in the pupa, when glycogen disappears; and if butter or olive oil is added to the diet, fat inclusions appear in earlier larval instars (46). Czihak (17) observed fat droplets in the haemocytes of *Carausius,* and suggested that these cells were concerned in food transport. They have not been observed in *Rhodnius* (78).

The best evidence for a nutritive function of the haemocytes derives from the study of wing development in Lepidoptera. For example, Zeller (87) considers that the haemocytes nourish the developing wing of *Ephestia,* not only through substances which they transport and give off, but through the adherence of a large number of these cells to the middle membrane of the wing; later they break down and their contents pass directly into the elongated processes of the epidermal cells. Large amounts of fat may be seen to be transferred directly to the cells in this way, and India ink particles carried by the haemocytes are likewise transferred to the epidermal cells.

As Lotmar (42) points out, the haemocytes of the honey bee play no part in the phagocytosis of histolysing tissues during metamorphosis. The great increase in their numbers at this time must therefore result from some other cause. Many of these abundant haemocytes are large and distended with vacuoles. Anglas (2), who observed these cells, supposed that they were ingesting materials liberated by the histolysis of the tissues, after the haemocytes had secreted the histolytic enzymes ("lyocytosis" hypothesis). But Lotmar favours the idea that they are transporting nutrients to the growing tissues.

The haemocytes are few in number on the bee larva; shortly before pupation they increase in size and number and fill with vacuoles which stain with azan. In the later stages of pupal development they are reduced in size once more and the vacuoles are more scanty. In the emerging bee they have disappeared. These cells accumulate around the actively growing organs, and Lotmar suggests that they are transporting nutrient substances of some undetermined nature.

In *Rhodnius,* as we have seen, the amoebocytes begin to increase in number, by the division of the proleucocytes, within a day after the meal which initiates the moulting cycle. On the fourth day there is a visible change in these cells: they are larger, and among the mucopolysaccharide inclusions there are large, watery vacuoles. It was suggested that this apparent secretory activity might be connected with the production of the factors stimulating growth (see below) (77). But it is worth considering whether this vacuolation, and the activities described by Zeller (87) and Lotmar (42), may not represent a first stage in the synthesis of the mucopolysaccharide inclusions that are going to form the connective tissues and basement membranes as described earlier in this review.

Phenol metabolism.—It is generally agreed that phenolic substances play an important part in the hardening and darkening of the insect cuticle and that these substances are mobilized during the later stages of the moulting cycle. There is some evidence that the haemocytes may be concerned in this process.

Poisson & Pesson (55) described blood cells with phenolic inclusions in the coccid *Pulvinaria*. These contain vacuoles which reduce osmic vapour and ammoniacal silver and give a brown colour with potassium iodate and potassium dichromate. In the larva of *Sarcophaga,* Dennell (18) observed that certain large oval haemocytes (35 to 40μ in length), termed by him "oenocytoids," are the only ones which give a deep blue colour with the "Nadi" reagent. If the blood is allowed to dry, these cells turn brown, as they do on the addition of catechol to exposed blood. They make their appearance in the blood late in the last larval stage as feeding ceases. Dennell suggests that they secrete tyrosinase into the blood and then disappear before pupation.

It is clearly these same cells which Jones (32) describes in the larva of *S. bullata* under the name of "spherule cells" (p. 5). They contain large spherical inclusions which turn vivid red-brown under methanol. They do not react with the G-nadi test and therefore do not contain cytochrome oxidase; but, as shown by Dennell, the M-nadi test is positive and they give a positive reaction on incubation with tyrosine. Probably they contain tyrosinase. Precisely how they intervene in the hardening process has not been established.

Growth stimulation.—In experiments on *Rhodnius* an attempt was made to see whether the haemocytes are involved in the chain of hormone secretions which lead to growth and moulting (77). It was found that "blockage" of the phagocytic amoebocytes by the injection of India ink, trypan blue or iron saccharate caused a prolonged delay in moulting. In the fourth-stage larva of *Rhodnius* moulting occurs about 14 days after feeding. Injection of India ink, etc., at 1 day, 2 days, or 3 days after feeding delayed moulting for several weeks. Injection at 4 days or later had no effect.

Further experiments confirmed the conclusion that blockage of the haemocytes does not affect the moulting process once the thoracic gland hormone has been secreted in adequate amount, but indicated that interference with the haemocytes in the earlier stages prevents the full activation of the thoracic gland by the hormone from the brain. Between 3 days and 4 days after feeding, as we have already seen, there is a visible change in the amoebocytes: at 4 days they become filled with watery vacuoles. It was suggested that these vacuoles might be the visible sign of some secretory or metabolic activity necessary for activation of the thoracic gland.

This was only one of the suggested explanations of the observed facts. Other possibilities were that interference with the haemocytes leads to removal or to inactivation of the brain hormone so that the effect on the thoracic gland is suppressed.

It is impossible to decide between these alternatives on present evidence. But looking at the problem broadly, one can say that injury of any kind, sufficiently early in the moulting process, causes a delay in growth. In any such injury the haemocytes are involved. Perhaps it is a mechanism which operates through the haemocytes that ensures the consequent arrest of growth; by the blockage of the haemocytes we may be bringing this machinery into action.

That the haemocytes might be capable of secreting factors that stimulate growth was suggested also by Shatoury & Waddington (67). They observed that after the cells (oikocytes) liberated by the lymph glands of *Drosophila* larvae appear below the imaginal discs (p. 1), visible changes in the activity of these organs begin. Shatoury & Waddington noted that these cells might "play some part in stimulating those processes of differentiation which bring the organs into a condition from which they can undergo metamorphosis." It is, of course, well known that changes of this kind are produced by the growth hormones and the observed activities of the oikocytes may be the result of hormone action rather than the cause of the growth changes.

Function of the oenocytoids.—No functions have been suggested for the oenocytoids. As seen in their typical form in *Rhodnius,* these are oval or rounded disc-shaped cells which always have a smooth outline with no trace of pseudopodial filaments. Stained with haematoxylin and eosin they show a clear, homogeneous, eosinophil cytoplasm (or with just a few minute vacuoles). In the fresh state they show uniform minute spherical vacuoles, mostly in contact with the cell surface, and the contents of these vacuoles stain with osmium and ethyl gallate (82).

Early in the moulting process the oenocytoids increase greatly in number and also in size. When the amoebocytes migrate to the basement membranes, they are accompanied by oenocytoids. Then in the later stages of moulting, during the period of cuticle formation, the latter are ingested by the amoebocytes (75). Sometimes as many as five or six of these cells may be seen within a single amoebocyte. Here they are broken down. For a time the remains of their pyenotic nuclei may be seen; finally all traces disappear.

Exactly the same process was described and figured by Poyarkoff (56): the eosinophil oenocytoids of *Galerucella* (Chrysomelidae) are ingested by the phagocytes during pupal development; a few persist in the young imago but soon disappear completely.

Although no evidence of what the oenocytoids are doing is available, they are clearly playing some important part in the metabolic processes concerned in growth and moulting.

LITERATURE CITED

1. Åkesson, B., *Arkiv Zool.*, **6**, 203-11 (1953)
2. Anglas, J., *Bull. sci. France et Belg.*, **34**, 1–111 (1900)
3. Arnold, J. W., *Can. J. Zool.*, **30**, 352–64 (1952)
4. Arvy, L., *Compt. rend.*, **235**, 1539–41 (1952)

5. Arvy, L., *Bull. soc. zool. France,* **78,** 158–71 (1953)
6. Arvy, L., *Proc. Roy. Entomol. Soc. (London),* [A]**29,** 39–41 (1954)
7. Arvy, L., Gabe, M., and Lhoste, J., *Bull. biol. France et Belge,* **82,** 37–60 (1948)
8. Arvy, L., and Lhoste, J., *Bull. soc. zool. France,* **69,** 230–35 (1944)
9. Arvy, L., and Lhoste, J., *Bull. soc. zool. France,* **70,** 144–48 (1945)
10. Baccetti, B., *Redia,* **41,** 75–104 (1956)
11. Baccetti, B., *Redia,* **41,** 259–76 (1956)
12. Beard, R. L., *Physiol. Zoöl.,* **23,** 47–57 (1950)
13. Benwitz, G., *Zool. Jahrb., Abt. Anat. u. Ontog. Tiere,* **75,** 311–78 (1956)
14. Berlese, A., *Zool. Anz.,* **23,** 441–49 (1900)
15. Berlese, A., *Zool. Anz.,* **24,** 515–21 (1901)
16. Cuénot, L., *Arch. zool. exptl. et gén.,* **9,** 593–670 (1891)
17. Czihak, G., *Mikrokosmos,* **45,** 169–71 (1956)
18. Dennell, R., *Proc. Roy. Soc. (London),* [B]**134,** 79–110 (1947)
19. Ermin, R., *Z. Zellforsch. u. mikroskop. Anat.,* [A]**29,** 613–69 (1939)
20. Grégoire, C., *Blood,* **6,** 1173–98 (1951)
21. Grégoire, C., *Biol. Bull.,* **104,** 372–93 (1953)
22. Grégoire, C., *Arch. biol. (Liége),* **66,** 103–48 (1955)
23. Grégoire, C., and Florkin, M., *Physiol. Comparata et Oecol.,* **2,** 126–39 (1950)
24. Hinton, H. E., *Sci. Progr.,* No. 168, 684–96 (1954)
25. Hollande, A. C., *Arch. zool. exptl. et gén.,* **6,** 283–323 (1911)
26. Hrdý, I., *Acta Soc. Entomol. Bohem. (Čsl.),* **54,** 305–11 (1957)
27. Huff, C. G., *Physiol. Revs.,* **20,** 68–88 (1940)
28. Janet, C., *Bull. soc. entomol. France,* 350–51 (1907)
29. Johannson, A. S., *Trans. Am. Entomol. Soc.,* **83,** 119–83 (1957)
30. Jones, J. C., *Ann. Entomol. Soc. Am.,* **46,** 366–72 (1953)
31. Jones, J. C., *Ann. Entomol. Soc. Am.,* **47,** 307–15 (1954)
32. Jones, J. C., *J. Morphol.,* **99,** 233–58 (1956)
33. Jones, J. C., and Tauber, O. E., *Ann. Entomol. Soc. Am.,* **44,** 539–43 (1951)
34. Kemper, H., *Z. Parasitenk.,* **5,** 112–37 (1932)
35. Kremer, J., *Z. mikroskop.-anat. Forsch.,* **2,** 536–81 (1925)
36. Lange, H. H., *Z. Zellforsch. u. mikroskop. Anat.,* **16,** 753–805 (1932)
37. Larchenko, K. I., *Bull. acad. sci. U.R.S.S., Sér. Biol.,* 203–10 (1946)
38. Lartschenko, K., *Z. Parasitenk.,* **5,** 679–707 (1933)
39. Lazarencko, T., *Z. mikroskop.-anat. Forsch.,* **3,** 409–99 (1925)
40. L'Helias, C., *Bull. soc. zool. France,* **78,** 76–83 (1953)
40a. Lhoste, J., *Arch. zool. exptl. et gén.,* **95,** 77–252 (1957)
41. Liebman, E., *Growth,* **10,** 291–330 (1946)
42. Lotmar, R., *Schweiz. Bienen-Ztg.,* **1,** 443–506 (1945)
43. Morgenthaler, P. W., *Mitt. schweiz. entomol. Ges.,* **26,** 245–57 (1953)
44. Muldrew, J. A., *Can. J. Zool.,* **31,** 313–32 (1953)
45. Munson, S. C., in *Insect Physiology,* 218–24 (Roeder, K. D., Ed., John Wiley & Sons, Inc., New York, N.Y., 1100 pp., 1953)
46. Munson, S. C., and Yeager, J. F., *Ann. Entomol. Soc. Am.,* **37,** 396–400 (1944)
47. Ochsé, W., *Rev. suisse zool.,* **53,** 534–37 (1946)
48. Oertel, E., *J. Morphol.,* **50,** 295–340 (1930)
49. Ögel, S., *Communs. fac. sci. univ. Ankara,* [C]**4,** 16–38 (1955)
50. Ohuye, T., and Horikawa, M., *Mem. Ehime Univ., Sect. II, (Sci.) Biol.,* **2,** 283–91 (1956)

51. Palm, N. B., *Arkiv. Zool.*, **3**, 195–272 (1952)
52. Pérez, C., *Arch. zool. exptl. et gén.*, **4**, 1–274 (1910)
53. Perttunnen, V., *Ann. Entomol. Fennici*, **21**, 78–88 (1955)
54. Poisson, R., *Bull. biol. France et Belg.*, **58**, 49–202 (1924)
55. Poisson, R., and Pesson, P., *Arch. zool. exptl. et gén., Notes et Rev.*, **81**, 23–32 (1939)
56. Poyarkoff, E., *Arch. anat. microscop.*, **12**, 333–474 (1910)
57. Riedel, F. A., *Ann. Entomol. Soc. Am.*, **39**, 298–303 (1946)
58. Rizki, M. T. M., *J. Exptl. Zool.*, **123**, 397–411 (1953)
59. Rizki, M. T. M., *J. Morphol.*, **100**, 437–58 (1957)
60. Rooseboom, M., *Arch. néerl. zool.*, **2**, 432–559 (1937)
61. Salt, G., *Proc. Roy. Soc. (London)*, [B]**144**, 380–98 (1955)
62. Salt, G., *Proc. Roy. Soc. (London)*, [B]**146**, 93–108 (1956)
63. Salt, G., *Proc. Roy. Soc. (London)*, [B]**147**, 167–84 (1957)
64. Savage, A. A., *Quart. J. Microscop. Sci.*, **97**, 599–615 (1956)
65. Schneider, F., *Vierteljahresschr. naturforsch. gesell. Zürich*, **95**, 22–44 (1950)
66. Shatoury, H. H. el, *Wilhelm Roux' Arch. Entwicklungsmech. Organ.*, **147**, 489–95 (1955)
67. Shatoury, H. H. el, and Waddington, C. H., *J. Embryol. Exptl. Morphol.*, **5**, 122–33 (1957)
68. Shatoury, H. H. el, and Waddington, C. H., *J. Embryol. Exptl. Morphol.*, **5**, 134–42 (1957)
69. Tauber, O. E., *Ann. Entomol. Soc. Am.*, **33**, 113–19 (1940)
70. Tauber, O. E. and Griffiths, J. T., Jr., *Proc. Soc. Exptl. Biol. Med.*, **51**, 45–47 (1942)
71. Toumanoff, C., *Rev. can. biol.*, **8**, 343–67 (1949)
72. Waterhouse, D. F., *Australian J. Sci.*, **13**, 25–26 (1950)
73. Webley, D. P., *Proc. Roy. Entomol. Soc (London)*, [A]**26**, 25–37 (1951)
74. Wigglesworth, V. B., *Nature*, **127**, 307–8 (1931)
75. Wigglesworth, V. B., *Quart. J. Microscop. Sci.*, **76**, 269–318 (1933)
76. Wigglesworth, V. B., *The Principles of Insect Physiology*, 51, 283–87 (Methuen, London, England, 544 pp., 1953)
77. Wigglesworth, V. B., *J. Exptl. Biol.*, **32**, 649–63 (1955)
78. Wigglesworth, V. B., *Quart. J. Microscop. Sci.*, **97**, 89–98 (1956)
79. Wigglesworth, V. B., *Quart. J. Microscop. Sci.*, **97**, 465–68 (1956)
80. Wigglesworth, V. B., *Ann. Rev. Entomol.*, **2**, 37–54 (1957)
81. Wigglesworth, V. B., *Proc. Roy. Soc. (London)*, [B]**147**, 185–99 (1957)
82. Wigglesworth, V. B. (Unpublished observations)
83. Wyatt, S. S., *J. Gen. Physiol.*, **39**, 841–52 (1956)
84. Yeager, J. F., *Ann. Entomol. Soc. Am.*, **32**, 49–57 (1939)
85. Yeager, J. F., *J. Agr. Research*, **71**, 1–40 (1945)
86. Yeager, J. F., and Munson, S. C., *J. Agr. Research*, **63**, 257–94 (1941)
87. Zeller, H., *Z. Morphol. Ökol. Tiere*, **34**, 663–738 (1938)

CULTURE OF INSECT TISSUES[1]

By M. F. Day and T. D. C. Grace
C.S.I.R.O., Canberra, A.C.T., Australia

INTRODUCTION

The first attempt to adapt Harrison's (49) method of tissue culture for insect tissues was made by Goldschmidt (38). Attempting to grow testicular follicle cells of a moth in a medium consisting of hemolymph, he observed maturation of the cells but no mitosis. In view of the usefulness of cultures of vertebrate cells in studies of embryology, morphogenesis, nutrition, genetics, pathology, cellular metabolism, and other fields, it was to be expected that comparable studies would be attempted with insects. However, the results were at first disappointing.

In this review an attempt will be made to determine the reasons for the apparent difficulties in successful insect tissue culture, to consider the results so far obtained, and to evaluate the application of tissue culture to entomological problems. No review covering these subjects has, to our knowledge, been published previously, although there are several valuable treatises on vertebrate and plant tissue culture (16, 82, 119, 120). The bibliography by Murray & Kopech (79) is invaluable for literature up to 1950. Many technical details are given in "Tissue Culture Methods" (87) and *An Introduction to Cell and Tissue Culture* (48).

It has been appreciated by many workers with vertebrate tissues that the term "tissue culture" is not particularly appropriate, as it has come to denote many lines of enquiry which might be better described as "cell culture" on the one hand or "organ culture" at the other extreme. Following Murray & Kopech (79), we shall define "tissue culture" as "the maintenance of isolated portions of multicellular organisms in artificial containers outside the individual for considerable periods of time." One aim of tissue culture is visualized as allowing cells or tissues to go through *in vitro* the activities they would be expected to undergo in the intact animal. Another goal, for the purpose of growing viruses in cells, for example, is to achieve a continuously multiplying, uniform strain of cells which can be readily cultured in chemically defined media.

HISTORICAL REVIEW

The history of insect tissue culture can conveniently be divided into three phases. During the first period, the majority of workers were interested in gametogenesis and employed simple mixtures of hemolymph and salts as the culture media. At best the cells completed their development,

[1] The survey of the literature pertaining to this review was completed in April, 1958.

TABLE I

OUTLINE OF STUDIES ON INSECT TISSUE CULTURE
1915 TO 1949

Date	Author and Reference	Species and Tissue	Medium	Result
1915	Goldschmidt (38)	cecropia moth spermatocytes	hemolymph	mitosis and gametogenesis
1916	Goldschmidt (39)	cecropia moth spermatocytes	hemolymph	survival
1916	Lewis (68)	grasshopper testes	dilute sea water and insect extracts	survival
1916	Lewis & Robertson (69)	grasshopper spermatocytes	Locke's solution plus peptone, sea water, sugar, etc.	mitosis and gametogenesis
1917	Goldschmidt (40)	(see 1915, Goldschmidt)		survival
1917	Glaser (36)	*Malacosoma* hemocytes	hemolymph, Locke's solution	survival and multiplication
1920	Collier (22)	*Musca* and *Calliphora* gut		
1924	Takakusa (106)	cecropia moth spermatocytes	pupal hemolymph	survival
1924	Paillot (81)	various insects	hemolymph	survival
1925	Schmidtmann (99)	*Dytiscus* muscle		short-period survival
1925	Chambers (19)	grasshopper spermatocytes	hemolymph	mitosis
1925	Lazarenko (67)	*Oryctes* hemocytes	larval hemolymph	mitosis and syncytial formation
1926	Murray (78)	cricket ovary	diluted sea water plus dextrose	survival
1928	Frew (30)	fly larva and imaginal discs	treated lymph	cell migration and partial differentiation

TABLE I (*continued*)

Date	Author and Reference	Species and Tissue	Medium	Result
1929	Bělař (10)	grasshopper spermatocytes	salt solution plus dextrose	mitosis and gametogenesis
1930	Kohring (57)	tissues of mosquito, grasshopper, and dragonfly	salt solutions	short-period survival
1930	Charin (20)	butterfly wing anlagen		cell migration and limited multiplication
1930	Baumgartner & Payne (8)	grasshopper spermatocytes	salt solutions	partial development
1935	Trager (110)	silkworm ovarian tissues	salts, sugars, hemolymph	migration and mitosis, survival for several weeks
1935	Taylor (108)	cockroach hemocytes	salt solution plus hemolymph	occasional mitosis
1935	Hibbard (52)	silkworm ovariole and other tissues	hemolymph, chicken plasma, and various salt solutions	survival for more than a month
1937	Pfeiffer (83)	*Corethra* nerve cells	mammalian media plus *Corethra* embryo extract	cell migration
1937	Takeda (107)	firefly light organ	salt solutions	short-period survival
1937	Trager (111)	silkworm ovarian tubule	salts, sugar, and hemolymph	migration of cells, partial development of *Nosema* parasites
1938	Trager (112)	mosquito imaginal discs	salt solution plus plasma plus albumin	differentiation but no mitosis, virus multiplication
1938	Wermel (117)	silkworm hemocytes	salt solution	differentiation and morphogenesis

TABLE I (*continued*)

Date	Author and Reference	Species and Tissue	Medium	Result
1939	Gottschewski & Fischer (42)	*Drosophila* eye discs	chick embryo extract, chick plasma, salts, and amino acids	some morphogenetic changes
1939	Pfeiffer (84)	*Corethra* ganglia		survival
1939	Fischer & Gottschewski (28)	*Drosophila* blood cells, wing and leg discs	(see 1939, Gottschewski & Fischer)	some mitosis and differentiation
1940	Stern (105)	*Drosophila* testis	salt solution	differentiation
1941	Gavrilov & Cowez (35)	mosquito salivary glands and gut cells	chick plasma, embryo extract, and mosquito extract	migration and mitosis
1942	Pfeiffer (85)	*Vespa* muscles	vertebrate plasma plus 5% peptone	cell migration
1943	Pfeiffer (86)	*Corethra* nerve tissue	salt solution	survival
1946	Carlson (17)	grasshopper neuroblasts	salts, glucose, and yolk	mitosis
1946	Millara (76)	various spp. hemocytes	hemolymph	short-period survival
1946	Arvy & Gabe (2)	*Forficula* hemocytes	hemolymph	mitosis and survival up to 24 hr.
1947	Lüscher (72)	*Rhodnius* epithelial cells and hemocytes	nymphal hemolymph	migration for 10 days
1947	Gaulden & Carlson (32)	grasshopper embryos	salt solutions	short-period survival
1948	Duryee (25)	grasshopper spermatocytes	salt solution	gametogenesis and cytokinesis
1949	Ris (93)	grasshopper spermatocytes	hemolymph	mitosis and gametogenesis
1949	Schmidt & Williams (97a)	silkworm spermatocytes	hemolymph	spermatogenesis

but generally without undergoing mitosis. Many interesting phenomena were, and will continue to be, discovered from such studies (98, 102).

During the second phase, attempts were made to improve the culture media, mainly by empirical testing. The media so devised were still largely influenced by knowledge of the composition of vertebrate tissue fluids and, even though survival of insect tissues for some days (110) and even weeks (70) was obtained, no significant cell growth resulted. Hartzell (50) has briefly reported the survival of insect tissues in hanging-drop cultures for up to three months.

There were many advances in the knowledge of the composition of arthropod tissue fluids (14) during the time that these studies were in progress, but only recently have these been employed in what we will call the third phase of development. In this, information concerning tissue fluids has been used in designing culture media particularly adapted to arthropods (for example, 44, 103, 123). With the further development of such media, tissue culture of arthropods should present no insoluble problems, but a major difficulty continues to be our inadequate knowledge of the chemistry of the insect tissue fluids. Continued subculturing has so far eluded all workers, although Grace (46) has succeeded in maintaining cells of promethea moth [*Callosamia promethea* (Drury)] pupae in culture for over 12 months.

Table I presents a chronological summary of results on insect tissue culture. [A similar compilation has been made by Loeb (69a).] It is believed to be fairly complete up to 1949. Later publications are discussed in detail elesewhere in this review.

CHARACTERISTICS OF TISSUES AND MEDIA FOR INSECT TISSUE CULTURE

It is important, when selecting an insect for tissue culture experiments, to keep in mind two main requirements: (*a*) the insect should be one in which the relevant tissues grow during larval life by increase in the number of cells, and (*b*) a comprehensive analysis of the hemolymph of the chosen insect should be available. Much work has been unrewarding because the media used were completely unsuitable for growth.

Tissues

Several characteristic differences between insect and vertebrate tissues are worth considering in relation to the problems of insect tissue culture.

Simple epithelia.—Stratified epithelia are never developed, even in large insects. Apparently compact organs, like the fat body, generally result from repeated folding of a single cell layer. The significance of this is that it is difficult to obtain fragments for tissue explants containing a density of cells comparable with those readily available for vertebrate tissue cultures. In the absence of "feeder cells" (91), tissue bulk has long been recognized as a necessity for growth in vertebrate tissue cultures, and it is

apparent that the small size of implants has in some instances been a factor in the failure of insect tissues to grow *in vitro*. Attempts to culture unadapted tissues from small fragments may succeed more readily if the tissues are confined at first to microtubes (96). Grace (44) has reported enhancement of growth resulting from an increase in the size of the inoculum, but it has been difficult to obtain the cell density thought to be optimal. Treatment with trypsin, to separate cells and thereby to facilitate their manipulation, is a standard technique with vertebrate cells but is not effective for insect tissues unless modified (see p. 32). Whatever technique is used for obtaining cells for the initiation of cultures, it is important that physical trauma be reduced as much as possible.

Tracheation.—The dependence upon diffusion through tracheae for gas exchange may well be a factor in limiting the size of insects and in promoting their extraordinary respiratory efficiency. Experiments to examine the effect of gas tension on the survival of hanging-drop preparations have not given any indication that gas tension within wide limits affects the growth of cells in culture (37). Thus, spermatogenesis proceeded at a normal rate at oxygen tensions between 21 per cent of an atmosphere (the normal tension in air) and 1 per cent. Decreasing the tension below 1 per cent caused inhibition of the process (101). The ovaries of the silkworm cultured in an atmosphere rich in oxygen showed no increase in growth or survival over those cultured in air (44), and the gas tensions in culture media may be altered without effect on the growth or survival of mosquito mid-gut tissues or of the malarial plasmodia infecting them (4). However, grasshopper neuroblasts are quite sensitive to high N_2 or CO_2 tensions (34). Ketchel & Williams (55) demonstrated a marked effect on spermatogenesis of a volatile substance which is not a normal constituent of air, is soluble in insect blood but is relatively insoluble in water. It appears to be a neutral, nonpolar substance, and its action has been compared to the effect of ethylene on fruit ripening. These interesting results emphasize the need for a consideration of the gas phase in the study of certain tissue cultures.

The membranes surrounding organs.—It has been suggested that a membrane encloses most organs. The permeability of this membrane may be such that the composition of the fluids reaching the cells bears little resemblance to the hemolymph. Untreated blood has not proved a satisfactory culture medium, even though it has often been used and gonads have survived in it for as many as 17 weeks (36). The blood contains large amounts of the enzyme tyrosinase which catalyzes the formation of melanin-like pigments, or substances associated with them, which are toxic to the tissues. Schmidt & Williams (98) found that the addition of phenylthiourea to cecropia moth [*Hyalophora cecropia* (Linnaeus)] blood was completely successful in inhibiting the formation of deleterious substances. The usefulness of the blood is further enhanced if it is heated at 60°C. for 5 min. to remove part of its protein content (123).

Embryonic determination.—The fact that eggs of many insects, for example those of Diptera and Hymenoptera, are "determined" in the embryological sense in respect to the outcome of any particular cell implies that the fate of each cell is unalterable even before eclosion takes place. All growth in many cells of dipterous larvae is the result of cell enlargement, and one could therefore not expect mitoses in these cells when they are explanted. On the other hand, eggs of Orthoptera and Odonata have great powers of regulation (58).

Attempts have been made to culture embryonic tissues, but so far with little success (43, 44). The gut and heart of cockroach embryos continued to contract for up to 54 days but there was no outgrowth of cells into the medium.

The embryo should be an ideal organism from which to obtain tissue for culturing. It is not only in a state of active growth, but all the hormones and other factors necessary for growth must be present either within it or in the yolk. Since it is widely appreciated that the tissues taken from embryos of vertebrates (chicks, mice, humans) grow well in culture, it is surprising that more success has not been obtained with insect embryos. Neuroblasts of grasshopper embryos have been extensively studied (17, 18, 32), and a medium for their cultivation has been provided (103).

Tissues for insect tissue culture.—No insect cell line is yet available, and it is therefore premature to suggest what tissues may be expected to yield the best results. The "melanotic tumors" of *Drosophila* have been grown in culture (31, 61 to 65) but not for any length of time. *Drosophila,* homozygous for one of the "female sterile" genes, have a high incidence of ovarian tumors (56), and cells from these may prove suitable for growth in culture.

Hemocytes would seem to be suitable cells to culture. Grace (43) found that the blood cells of the silkworm larva formed a syncytium within 20 hr. and that this survived for 6 days in the media he used. Schmidt & Williams (98) reported that hemocytes of cecropia larvae flattened, formed syncytial tissue, and survived unchanged in blood of diapausing pupae for more than 30 days. However, in blood from developing adults the syncytia developed after 5 or 6 days and a few mitoses were still present after 2 weeks. Masses of small cells appeared which did not correspond with any of the various types of blood cells, and mitoses continued in them for some time. Trager (110) was the first to demonstrate that the inner envelope of the ovarian follicle of caterpillars provided the cells which grew best in culture. Subsequent work with the silkworm has confirmed that this tissue grows better in culture than any others tested, but the reasons for this are completely obscure. Even the origin of the tissue is not certainly known; it may originate from sedentary hemocytes.

Imaginal discs provide an abundance of cells undergoing mitosis but they too seem to be completely determined embryologically (24, 30, 66).

Organ culture.—For many purposes it is desirable to maintain entire

organs in culture rather than isolated cells and tissues. Thus, Ball (6) wished to observe developing malarial oöcysts on the gut wall of the mosquito. Although the medium employed was adequate to maintain contractions in the mosquito hind-gut for almost 5 weeks, the oöcysts did not develop.

The examination of regenerating cells under high magnification has been carried out by a technique developed by Lüscher (72, 73). A leg of a freshly fed fifth-instar nymph of *Rhodnius prolixus* Stål was amputated at the tibia and bathed in physiological salt solution; a fine glass capillary tube was introduced into the open end of the limb. Up to 1 mm. of the tube was covered with a network of epithelial cells within 24 hr. Beckel (9) attempted to study egg development in mosquitoes by culturing ovaries in hanging drops. Ovaries of *Aedes aegypti* (Linnaeus) survived for up to 60 days, but no development occurred.

Media

A major factor in attaining successful insect tissue culture is the nature of the suspending medium.

Basic biochemical unity.—Animals exhibit a remarkable uniformity in chemical composition, and many of the fundamental metabolic pathways of insects and vertebrates are similar (29). The synthetic diets suitable for raising germfree animals are essentially the same for insects and vertebrates (97). It would, therefore, be surprising if differences were to appear, at the metabolic level, which would render the tissue culture media developed for vertebrates totally unsuited to the requirements of insect tissues. In fact, Loeb & Schneiderman (70) have obtained survival of the silkworm epidermis for 35 days in the chemically defined vertebrate medium (TC 199) of Morgan, Morton & Parker (77).

On the other hand, the extraordinary sensitivity of different tissues, even from the same species, to slight variations in the medium is one of the most outstanding features of the culture of vertebrate cells. Analyses of insect hemolymph have shown it to be quite unlike that of vertebrate fluids in, for example, ionic balance, the high amino acid and organic phosphate content (121), and in other respects. Furthermore, some metabolic pathways in insects do differ from those encountered in vertebrates. These facts have prompted the suggestion that insect tissue culture will present no unusual difficulties once media of the appropriate composition are developed. Not very much progress toward this aim has been made, but Wyatt's medium (123) is a substantial improvement over previous media for silkworm tissues, and it is suitable for some other Lepidoptera.

There is a difficulty in comparing dietary requirements with the components of a complete *in vitro* culture medium, for the synthetic ability of the intact animal may be significantly more complete than that of individual tissues. Thus, it is probable that growing tissues may require growth

factors over and above those essential to the intact insect from which they were derived. But it is also possible that tissues may be called upon to perform fewer synthetic functions than the intact animal and may be able to dispense with certain substances in the medium. For example, cholesterol is probably an essential growth factor for all insects (113), but Grace (44) found that its presence had no apparent effect on silkworm cells in Wyatt's medium. However, such discussion cannot usefully be carried very far, for the permeability of the insect cell membrane is unknown and a substance may seem to be of no consequence in a medium when, in fact, the reason for its apparent inactivity is that it cannot reach the sites of activity. Such an hypothesis may explain the interesting observation of Grace (44) that trehalose, the blood sugar of the silkworm larva, was utilized only when endocrine-organ extracts were incorporated in the medium.

"Conditioned" media.—The use of conditioned media has proved of great value with vertebrates for growing cells which have otherwise proved difficult to culture. The media may be modified in several ways (91, 96). Although some attempts have been made to grow insect tissues in similarly modified media (44) little success has been obtained. The feeder layer technique of Puck and his associates (91) has not so far been employed with arthropod tissues.

Ionic composition.—Although living organisms possess a considerable degree of uniformity in chemical composition, there are many basic differences in lymph composition between vertebrates and insects. The majority of "balanced salt solutions" designed for use with insects have been empirical mixtures based on vertebrate physiological solutions rather than on the composition of hemolymph.

Insect tissues are less sensitive to ionic balance than those of vertebrates (e.g., 7), and their prolonged survival in media such as TC 199 (e.g., 9, 70) attests to this. Lewis & Robertson (69) and Stern (105) cultured grasshopper spermatocytes and the testes of *Drosophila*, respectively, in diluted sea water and obtained some development of the tissues. Others, for example, Lewis & Robertson (69) and Glaser (36), used Locke's solution—originally designed for maintaining frog tissues—or modifications of it. But, it is clear that physiological salt solutions for use with insects should be based on the composition of insect fluids. One reason is that the ionic composition of the hemolymph of different species differs so greatly that it is impossible to predict the composition of that of any species from investigations on related ones. Furthermore, marked changes may occur during the molting cycle (53).

Salt mixtures adequate for a number of insects are now available [e.g., see Buck (14)]. For the silkworm, Wyatt's medium (123) is suitable, even though the Na/K ratio of this medium is very different from that of silkworm hemolymph (109). On the basis of analysis of the inorganic constituents of mosquitoes, Clark & Ball (21) considered that the salt solutions

developed for mosquitoes by Bradford & Ramsay (12) and by Hayes (51) should both be satisfactory, even though they differ considerably from one another.

Shaw (103) analyzed the cations of grasshopper embryos and designed a medium with a Na/K ratio of 1.66:1 and a Ca/Mg ratio of 2:1. He demonstrated that tonicity of the solution had a marked effect on the number of mitoses in neuroblasts. The composition of the solution in which cockroach heartbeat is maintained longest is NaCl 11.0, KCl 1.4, and $CaCl_2$ 1.1 gm. per l. (71).

No medium for insect tissue culture has included any metals other than Na, Mg, Ca, Fe, and K. While these are certainly the metals of greatest importance, certain other trace elements deserve closer study, as they do for vertebrates (116).

Energy sources.—Glucose is the principal energy source in the majority of vertebrate culture media, and experience indicates that it is utilized by many insect tissues. However, trehalose is the principal sugar of hemolymph of many insects (122). When trehalose replaced glucose in culture media, silkworm tissues did not show improvement (44). Trager (110) found maltose more effective for silkworm tissue culture and sucrose and glucose less suitable. Wyatt (123) reported that a mixture of glucose, fructose, and sucrose in the approximate proportions 4:2:1 was optimal. Ball (6) used fructose as the principal sugar for mosquito tissues.

It is apparent that no generalizations are yet warranted concerning energy sources for insect tissues, but it would seem most logical to use the accepted di- and monosaccharides for insect media until others have been shown to be superior. The concentration of carbohydrates should be as high as possible without causing hypertonicity of the medium.

Amino acids.—High concentrations of amino acids (50 times that of human serum) are characteristic of insect hemolymph, and mixtures of amino acids are probably essential ingredients of culture media. Qualitative data on amino acids in hemolymph (14) are less useful than quantitative data for designing media but the latter are available for relatively few insects. Hackman (47) has provided data for a blowfly larva, and Wyatt, Loughhead & Wyatt (121) for the silkworm. Because marked changes in composition occur during development, the mixtures used in culture media must be to some extent empirical. Surprisingly high concentrations of certain amino acids may be added to media one at a time without deleterious effects on vertebrate tissues (13). However, it is most desirable that a quantitative study of the free amino acids in hemolymph should precede work on culture media.

A significant contribution to the design of media was made by Shaw (103) when he substituted amino acids for anions, particularly chloride. In his medium, sodium and potassium glutamate provided buffering and energy sources and greatly increased the survival period of cells—but did not affect the level or duration of mitotic activity.

Peptone has been reported to stimulate growth of vertebrate tissues, and Loeb & Schneiderman (70) increased the survival times of a number of tissues by the addition of 2-per-cent Bacto-peptone to their medium. Casein hydrolysate has also been used (114).

Other organic acids.—The high proportion of organic acids in most insect hemolymph examined suggests that they are significant intermediary metabolites. Only malate was stimulatory when tested alone; but a mixture of malate, ketoglutarate, succinate, and fumarate resulted in marked stimulation of growth (123). The effect of comparable mixtures should be studied in the development of media for other insects. Wyatt (123) found that citrate and lactate were not beneficial in culture media. Shaw (102) found ethanolamine phosphoric acid in grasshopper embryos and thought that it might be an important constituent of media for embryonic tissues. But its addition to culture media was without effect (103).

Vitamins.—It has been known for many years that insects require certain of the B vitamins necessary for vertebrates, and some may have additional requirements, e.g., carnitine (113). Wyatt's (123) medium lacked these "growth" factors. Vitamins in Eagle's (26) mixture for vertebrate cells include biotin, folic acid, nicotinamide, panthothenate, pyridoxal, thiamine, riboflavin and choline; all are necessary for aseptic culture of *Drosophila* larvae (97), and most of these are included in TC 199. Grace (44) has examined the effect of incorporating these and also nicotinic acid, *p*-aminobenzoic acid and *meso*-inositol in Wyatt's medium. An improvement in the appearance of the cells resulted, but no effect on the mitotic rate or on cell survival was noted; however, the vitamins were not tested in a wide range of concentrations.

Certain sterols are essential dietary constituents of insects, but Grace (44) found no effect on tissue cultures of 0.03 mg. per ml. of cholesterol.

Nucleic acids.—Waymouth (116) reports that there is no evidence that purines or pyrimidines or their polymers are required as nutrients for vertebrate cells, and Grace (44) found that neither ribose nucleic acid nor deoxyribose nucleic acid had any effect on the growth of silkworm tissues in culture.

Adenosine triphosphate.—This important metabolite is essential in many metabolic processes. It was incorporated into Wyatt's modified medium at concentrations of 2.4, 4.8, and 9.6 μg./ml. No effect on either growth or survival was observed at any of the concentrations used (46).

Hormonal environment.—That the growth and metamorphosis of insects are under the control of hormones has been known for a long time. The impression has been formed that insect tissues are more dependent upon hormones than are the tissues of vertebrates. As Bodenstein (11) has pointed out, "The various hormone systems are at all times in the postembryonic life of insects in a definite balance which is stage and time specific, . . . [and] any disturbance of this balance may lead to severe structural and metabolic alterations." Schmidt & Williams (98) showed that blood from

developing adult moths stimulates several types of cells to divide. These observations have suggested the need for the addition of one or more of the hormones of the corpus allatum, the prothoracic gland, or the brain to insect tissue culture media. The prothoracic gland hormone has been obtained in crystalline form (ecdyson), but when added to cultures of either diapausing or developing insect ovary it had no significant effects; a change in cell morphology was produced, however, in cultures of *Cynthia* pupal leg tissues by 2 μg. per ml. of ecdyson in aqueous solution (100). Considerable stimulation of mitoses in silkworm cultures resulted when an extract of either endocrine organs or ovaries was added to the media (44). A hormone extract made from the ring glands of last-stage larvae of *Lucilia cuprina* (Wiedemann), and prothoracic glands of last-stage nymphs of the American cockroach in Wyatt's medium increased the mitotic activity of the cells of the ovaries of the silkworm very markedly. A similar stimulation resulted when an extract made from ovaries of the fifth-instar silkworm was added to the medium. It is apparent that much further work is necessary on the role of hormones in insect tissue culture.

Tissue extracts.—Various tissue extracts, from both vertebrates and insects, have been used in attempts to increase the growth of insect tissues *in vitro*. They were first employed by Lewis (68), who found that the follicle cells and isolated sperm cells of grasshopper testes remained healthy and continued to divide in a medium of diluted sea water containing 20 per cent of grasshopper bouillon. Wyatt (123) and Grace (44) used embryo extracts of the silkworm in a medium containing 22 amino acids, organic acids, sugars, and salts in attempts to grow the ovary tissue of the silkworm. Some improvement in the cultures was obtained, especially when the extract was placed in boiling water for 2 min. to remove some of the protein. The frequency of mitoses in grasshopper neuroblasts was increased as the quantity of yolk was increased, up to an amount equivalent to one quarter of that occurring in the egg (33).

The wing, leg, and eye imaginal discs of *Drosophila* larvae have been cultured in a medium containing salts, amino acids, vitamins, chicken plasma, and embryo extract. Some development was obtained (28, 42). Trager (112) kept the leg, antennal sacs, brain and adjacent head tissue, and the ovaries of *Aedes aegypti* (Linnaeus) surviving for as long as 2 weeks in a medium containing salts, heparinized plasma, egg albumen digest, and phosphate buffer. The ovaries showed continuous muscle contractions for 20 days. Trager (110) also obtained limited growth of ovarian tissue of the silkworm in a medium of salts, glucose, egg albumen digest, and silkworm hemolymph.

Some workers have incorporated insect tissue extracts in the media along with the vertebrate extracts. The medium of Gavrilov & Cowez (35) included an extract made from mosquito larvae, in addition to salts, chicken plasma, and chick embryo extract. When the salivary glands and intestinal cells of two species of mosquito were cultured in this medium they sur-

vived, with some migration and growth, for 20 days. In a series of experiments using many media containing salts, amino acids, vitamins, cystine, glutathione, cholesterol, chick embryo extract, chicken plasma, and either adult or embryonic insect extracts, Grace (43) cultured nine tissues from four species of insects. He was unable to keep the tissues alive for longer than 6 days, and no mitoses and little migration were observed. One embryo of the American cockroach in a medium of salts, glucose, chick-embryo extract, chicken plasma, and cockroach embryo extract survived (with no growth) for 57 days, during which time movements of the head, heart, and gut were observed. Demal (24) used an extract from fly pupae together with chick embryo extract to culture the optic lobes, optic discs, and leg imaginal discs of *Drosophila* and *Calliphora*. The discs developed to a stage in which the legs showed segmentation and pigment was produced in the eyes. These cultures survived for 4 to 5 days. Friedman & Burton (31) have used extracts of *Drosophila* in a medium for the culture of tumors.

The use of vertebrate extracts either alone or with insect extracts has, on the whole, proved unsuccessful in increasing growth *in vitro*. The presence of insect extracts increased the mitotic activity of the cells to a small extent. There is some evidence that the growth of insect tissues cultured *in vitro* is inhibited by the presence of the certain proteins. As very few of the extracts employed had the protein removed, this could be a factor in the poor results obtained.

Hemolymph.—A number of authors have used hemolymph as a medium for tissue culture (see Table 1). Its value is enhanced after heat treatment (44, 114, 123). Methods other than heat treatment, such as those used in the preparation of vertebrate serum for culture media, might be found useful.

Optimal media.—In the design of media for cultivation of tissues of insects which have not previously been studied, a review of the literature on hemolymph composition will provide an essential starting point. The information available up to 1953, is excellently summarized by Buck (14). Useful contributions published since 1953 include a survey of the inorganic constituents of the blood of the cockroach (3), a discussion of isolated heart activity of a grasshopper and a moth in physiological salt solutions (7), and quantitative amino acid analyses of *Calliphora* hemolymph (47). The ideal approach to the design of media is to obtain dose-response curves for all major components of the medium under conditions in which interactions between them are reduced to a minimum.

We have insufficient data to outline the composition of adequate media for most insects. The composition of media devised for maintaining *in vitro* grasshopper neuroblasts is given by Shaw (103), for mosquito midgut by Ball (6), and for silkworm ovarian tissues by Wyatt (123). Grace (44) recommends adding to the latter medium vitamin B components and extracts of both endocrine organs and larval ovaries or extracts of one

of these. We suspect that there may be as yet unrecognized growth factors which must be added to media to ensure optimal growth of insect tissues. [See also Morgan (76a)].

OTHER TECHNICAL ASPECTS OF TISSUE CULTURE

Considerations other than the nature of the tissues and the media will be considered in this section. It is general experience that cells are not at first adapted to the conditions imposed in culture. Not infrequently the majority of cells set up in a culture degenerate, but from a few surviving and better adapted cells a strain may develop which may be subsequently subcultured.

Temperature

Insects have never evolved a homeothermic mechanism and habitually maintain temperatures lower than those of vertebrates. The optimum temperature for tissue growth should, therefore, be determined. It is probable that most insect tissues will grow poorly, if at all, at 37°C., whereas many vertebrate cells will not grow at room temperature. The temperatures generally utilized for insect tissue have been "room temperature," or if more closely specified, between 24° and 28°C. Vago & Chastang (114) used 30°C. with success. Ball (6) found it advantageous to keep cultures at 17° to 18°C., for the first 24 hr. before maintaining them at room temperature.

Culture Flasks

The relative merits of various types of culture vessels have been reviewed by Parker (82) and Cameron (16).

Hanging drops.—Many successful tissue culture experiments have employed hanging-drop preparations. The main drawbacks to the use of these include the technical difficulties attending medium replacement and the rapid accumulation of metabolites and waste products in the small volume used. Thus, frequent change of the medium is necessary. Perhaps the main advantage of this method is that it allows critical microscopic study of individual cells. Ovarian tissues of the moth *Callosamia promethea* (Drury), which were cultured for short periods in flasks and roller tubes and then transferred to hanging drops, have grown for 165 days and survived for at least 55 weeks even when the medium was changed only once every 7 days (46). Loeb & Schneiderman (70) obtained survival of the wing and antennal hyodermis of *Samia cynthia* (Drury) and *Antheraea polyphemus* (Cramer) for 6 weeks in the medium TC 199 plus 2-per-cent peptone and phenylthiourea. Wyatt (123) also used hanging drops successfully. A single explant (silkworm ovary) was placed in 0.005 ml. of medium on a glass cover slip, inverted over a 0.8 mm. depth depression slide and the cover slip sealed with vaseline. Schneiderman, Ketchel & Williams (101) employed "sitting drops" to study metabolic inhibitors of spermatogenesis of the cecropia moth. About 20 $mm.^3$ drops were pipetted onto a

glass plate and a second plate placed over the first, being separated from it by a brass ring. The chamber was sealed with melted paraffin.

Carrel flasks.—The use of these flasks has not been as successful for the growth of insect tissues as might have been anticipated. They permit the use of a large amount of medium, a large number of explants, and the gas phase is quite large. The main difficulty appears to be that the number of cells per unit of volume has been too low.

Roller tubes.—The advantages of this method are similar to those of the Carrel flask, but in addition the continual rotation of the tubes ensures a constant change of aerated medium. The method has been used by several workers (44, 114, 123).

Shaking flasks.—Although this technique has proved very successful for growing large amounts of vertebrate tissue (27), it has been used only by Grace (45) for the growth of insect tissues. Ovaries of diapausing promethea moth pupae were established in 0.7 ml. of medium in a 25-ml. Erlenmeyer flask and shaken at about 40 r.p.m. for 8 days. A large number of cells were obtained which remained healthy when transferred to hanging drops; many mitotic divisions were observed.

Substrates

Cells vary in their ability to grow on glass, cellophane, and other substrates. Agar, perforated cellophane, chicken plasma clots, and cellulose gel have all been used in an attempt to increase the growth of the tissues, but none have proved advantageous (46).

Hydrogen Ion Concentration

Tissues are sensitive to changes in the pH of the medium. Most media are well buffered, but changes in pH are often encountered during cultivation. Phenol red (0.005 per cent) is frequently incorporated to assist in detecting such changes. Ball (4) attempted to control the pH of media by passing sterile CO_2-free air through the liquid daily. If media were too alkaline he passed gas containing 3-per-cent CO_2 through them until they showed a neutral reaction. Attempts to use a variety of buffer systems have not been outstandingly successful.

Antibiotics

The incorporation of antibiotics is widely practiced to assist in the maintenance of sterility. It is particularly useful when nonsterile materials, such as virus suspensions, must be added to cultures. Little information is available on the sensitivity of insect tissues to antibiotics; Grace (43) found no effects from chloromycetin and sodium sulfadiazine. Grace (45) added penicillin and streptomycin to the medium at a concentration of 0.03 mg./ml. and 0.1 mg./ml. respectively and observed no adverse effects on the cells. Ball (4) found 100 to 200 units of penicillin per ml. of medium effective in controlling contamination, but Ball (6) used 330 to 400 units.

Contamination of cultures by molds frequently causes more difficulty than bacteria. Ball (6) controlled molds with 0.033 mg. per ml. of Actidione but found this concentration resulted in some cytopathologic changes in the mosquito tissues. Maramorosch (74) found that mycostatin satisfactorily prevented mold growth.

Methods of Sterilization of Insects

Sterility in cultures presents no great difficulty especially if penicillin and streptomycin (or other suitable antibiotics) are used in the medium. The surfaces of insects have been satisfactorily sterilized with potassium permanganate (74), mercuric chloride (41, 98, 110), 70-per-cent alcohol (46), hyamine (123), or hexylresorcinol (4).

Sensitivity to Growth Inhibitors

One of the main reasons tissues often fail to grow in culture is the presence of growth inhibitors. It has been found necessary to use metalfree distilled water in culture media, to take special care with the washing of glassware, and to ensure that rubber stoppers, etc., are free from toxic or inhibitory substances. Details of these precautions are given in Parker (82), Cameron (16), and Hanks & Scherer (48).

Separation of Cells

Trypsinization to obtain cells for subcultures is routine practice with vertebrate tissues. However, the techniques used are deleterious to insect tissues and a completely satisfactory method for separating insect tissues is, with two exceptions, not yet available. Grasshopper neuroblasts have been satisfactorily separated in a solution of trypsin and hyaluronidase with gentle agitation (104). Liberation of undamaged cells from the integument of the thoracic segments of *Peridroma margaritosa* (Haworth) was accomplished by Martignoni, Zitcer & Wagner (75) with an extract rich in hydrolytic enzymes, prepared from the hepatopancreas and crop of the snail *Helix aspersa* Müller.

Availability of Media

Many culture media for vertebrate tissues are available commercially in a ready-to-use form, and certain of these have been used in work with insect tissues (9, 70). While no media specifically designed for insect tissues are available, many of the ingredients may be obtained in a convenient form (e.g., vitamin or amino acid mixtures). For details, see Pomerat (87).

Measurement of Growth

It is important to appreciate the difficulties of establishing criteria for growth in tissue cultures. Many authors have used purely subjective criteria and simply based their views on the appearance of cells. Such methods can be misleading and are giving way to objective methods such as counting nuclei (95), or nucleic acid determinations (115).

THE USES OF INSECT TISSUE CULTURE

It is no exaggeration to say that work with insect tissues could follow most of the many lines of work for which the technique has proved useful for vertebrates and plants (119).

Morphogenesis

Several authors have studied the development of imaginal discs of Diptera (24, 30, 59, 60, 66, 105). Limited differentiation, but no cell division, was observed, probably because inadequate media were employed in all these studies.

Hemocyte cytology and changes in hemocytes have been studied by several authors. Again, the techniques used have generally been inadequate, although there are a number of reports of survival of hemocytes for some weeks (2, 36, 50, 67, 108).

Many details of gametogenesis have been elucidated by tissue culture techniques (10, 40, 69), especially when combined with phase-contrast cinematography (25, 93). Carlson and his co-workers (18) have made considerable progress toward an understanding of the mechanism of mitosis in the grasshopper neuroblast. Improved media for the latter preparation have recently become available (102). Lüscher (73) has employed a technique of "*in vivo* tissue culture" to study regeneration in the insect leg.

Cellular Nutrition and Metabolism

Vertebrate tissue cultures have contributed considerably in the fields of cellular nutrition and metabolism, but only a beginning has been made with cultures of insect tissues. Beckel (9) planned to study the nutritional requirements of the mosquito ovary, and Loeb & Schneiderman (70) the effects of hormones and nutritional factors on growth. Bucklin (15) has attempted to study the diapause of grasshopper embryos in culture. Carlson (17) described changes in cytoplasmic viscosity during mitosis in the grasshopper neuroblast. Kuroda & Tamura (63, 64) have investigated the effects of iron and copper on the growth of *Drosophila* melanotic tumors in tissue culture.

Pharmacology

Kuroda & Tamura (62) reported that melanotic tumor development in tissue culture of *Drosophila* was inhibited by 1 m*M* phenylthiocarbamate added to the synthetic medium.

Hartzell (50) has used insect tissues in culture for the assay of insecticides, and Schmidt & Williams (98) have developed the technique for the assay of the prothoracic gland hormone. The effects of various substances on spermatogenesis *in vitro* have been studied (54, 101). Gaulden & Carlson (32) have examined the effects of colchicine on cell division and on the mitotic spindle in the grasshopper embryo. The use of insect tissue cultures in pharmacological investigations is certain to become a field of

greater activity when the techniques are more readily standardized. Some of the difficulties of such an application have been discussed by Pomerat & Leake (88). For example, in vertebrates it is known that the intact animal may react differently to its tissues in culture.

Pathology

Perhaps the greatest current interest in insect tissue cultures arises from the spectacular results coming from the application of the technique to the culture of viruses and the production of vaccines (94). In 1917, Glaser (36) reported an attempt to grow a polyhedrosis virus *in vitro*. Trager (110, 111, 112) succeeded in growing a polyhedrosis virus in silkworm tissue cultures and the virus of western equine encephalitis in surviving mosquito tissues; he obtained also partial development from amebulae to planonts of the microsporidian *Nosema bombycis* Naegeli in a silkworm tissue culture. Aizawa (1) has demonstrated a "dark phase" following infection of silkworm tissue by the polyhedrosis virus. Tissue cultures are bound to assist in the vexed problems of "latency" in virus infections, and Grace (46a) has observed that polyhedroses occur in tissues of tussock-moth larvae subjected to a sudden change of medium. Maramorosch (74) demonstrated the completion of the extrinsic incubation period in surviving leafhopper tissues and the possibility of virus multiplication, but found no indication of the tissue in which multiplication took place, the main difficulty being in the lack of a satisfactory quantitative assay of virus titer. Ragab (92) and Ball (5, 6) have developed the culture of the mosquito gut in attempts to cultivate oöcysts of the malarial parasite. Friedman & Burton (31) have studied a tumor-inducing factor in *Drosophila* tissue cultures.

Price (89) has reported that Japanese B virus lost its infectivity for a time when grown in mosquito tissue culture. His data support the hypothesis that this virus goes through a noninfectious stage in mosquito tissues (90). Weyer and collaborators have grown Rickettsiae in arthropod tissues (80, 118). These examples illustrate some of the uses of tissue culture in pathology. Much remains to be done.

LITERATURE CITED

1. Aizawa, K. (Personal communication, 1957)
2. Arvy, L., and Gabe, M., *Compt. rend. soc. biol.*, **140,** 787–89 (1946)
3. van Asperen, K., and van Esch, I., *Arch. néerl. zool.*, **11,** 342–60 (1956)
4. Ball, G. H., *Am. J. Trop. Med.*, **27,** 301–7 (1947)
5. Ball, G. H., *Am. J. Trop. Med.*, **28,** 533–36 (1948)
6. Ball, G. H., *Exptl. Parasitol.*, **3,** 355–67 (1954)
7. Barsa, M. C., *J. Gen. Physiol.*, **38,** 79–92 (1954)
8. Baumgartner, W. J., and Payne, M. A., *Science*, **72,** 199–201 (1930)
9. Beckel, W. E., *Nature*, **177,** 534–35 (1956)
10. Bělař, K., *Arch. Entwicklungsmech. Organ.*, **118,** 359–484 (1929)
11. Bodenstein, D., in *Aspects of Synthesis & Order in Growth* (D. Rudnick, Ed., Princeton Univ. Press, Princeton, N.J., 1955)
12. Bradford, S., and Ramsay, R. W., *Federation Proc.*, **8,** 15–16 (1949)
13. Brues, A. M., Subbarow, Y., Jackson, E. B., and Aub, J. C., *J. Exptl. Med.*, **71,** 423–38 (1940)
14. Buck, J. B., in *Insect Physiology*, Chap. 6 (K. D. Roeder, Ed., John Wiley & Sons, Inc., New York, N.Y., 1953)
15. Bucklin, D., *Anat. Record*, **117,** 539 (1953)
16. Cameron, G., *Tissue Culture Technique*, 2nd ed. (Academic Press, Inc., New York, N.Y., 191 pp., 1950)
17. Carlson, J. G., *Biol. Bull.*, **90,** 109–21 (1946)
18. Carlson, J. G., *Science*, **124,** 203–6 (1956)
19. Chambers, R., *Cellule*, **35,** 105–24 (1925)
20. Charin, N., *Trav. inst. recherches sci. univ. Voroneje*, **4,** 103–23 (1930)
21. Clark, E. W., and Ball, G. H., *Physiol. Zoöl.*, **27,** 334–41 (1954)
22. Collier, W. A., *Z. wiss. Insektenbiol.*, **16,** 1–5 (1920-21)
24. Demal, J., *Ann. sci. nat. Zool. et biol. animale*, **18,** 155-61 (1956)
25. Duryee, W. R., *J. Wash. Acad. Sci.*, **38,** 31 (1948)
26. Eagle, H., *Science*, **122,** 501 (1955)
27. Earle, W. R., Bryant, J. C., Schilling, E. L., and Evans, V. J., *Ann. N.Y. Acad. Sci.*, **63,** 666–82 (1956)
28. Fischer, I., and Gottschewski, G., *Naturwissenschaften*, **27,** 391–92 (1939)
29. Florkin, M., *Biochemical Evaluation* (S. Morgulis, Trans., Academic Press, Inc., New York, N.Y., 157 pp., 1949)
30. Frew, J. G. H., *J. Exptl. Biol.*, **6,** 1–11 (1928)
31. Friedman, F., and Burton, L., *Cancer Research*, **16,** 1059–61 (1956)
32. Gaulden, M. E., and Carlson, J. G., *Genetics*, **32,** 87 (1947)
33. Gaulden, M. E., and Kokomoor, K. L., *Proc. Soc. Exptl. Biol. Med.*, **90,** 309–14 (1955)
34. Gaulden, M. E., Nix, M., and Moshman, J., *J. Cellular Comp. Physiol.*, **41,** 451–70 (1953)
35. Gavrilov, W., and Cowez, S., *Ann. parasitol. humaine et comparée*, **18,** 180–86 (1941)
36. Glaser, R. W., *Psyche*, **24,** 1–7 (1917)
37. Glaser, R. W., *J. N. Y. Entomol. Soc.*, **26,** 1–3 (1918)
38. Goldsmidt, R., *Proc. Natl. Acad. Sci. U. S.*, **1,** 220–22 (1915)

39. Goldschmidt, R., *Biol. Zentr.*, **36,** 161-67 (1916)
40. Goldschmidt, R., *Arch. Zellforsch,* **14,** 421–50 (1917)
41. Goodchild, A. J. P., *Nature,* **173,** 504–5 (1954)
42. Gottschewski, G., and Fischer, I., *Naturwissenschaften,* **27,** 584 (1939)
43. Grace, T. D. C., *Nature,* **174,** 187–88 (1954)
44. Grace, T. D. C., *Australian J. Biol. Sci.,* **11,** 401–17 (1958)
45. Grace, T. D. C., *J. Gen. Physiol.,* **41,** 1027–34 (1958)
46. Grace, T. D. C. (Unpublished material)
46a. Grace, T. D. C., *Science,* **129,** 249–50 (1958)
47. Hackman, R. H., *Australian J. Biol. Sci.,* **9,** 400–5 (1956)
48. Hanks, J. H., and Scherer, W. F., *An Introduction to Cell and Tissue Culture* (Burgess Publishing Co., Minneapolis, Minn., 123 pp. 1955)
49. Harrison, R. G., *Proc. Soc. Exptl. Biol. Med.,* **4,** 140–43 (1907)
50. Hartzell, A., *Ann. N.Y. Acad. Sci.,* **58,** 1157 (1954)
51. Hayes, R. O., *J. Econ. Entomol.,* **46,** 624–26 (1953)
52. Hibbard, H., *Bull. Mount Desert Is. Biol. Lab.,* **37,** 16–18 (1935)
53. Hoyle, G., *Nature,* **178,** 1236–51 (1956)
54. Ketchel, M. M., Feder, N., and Schneiderman, H. A., *Anat. Record,* **111,** 518 (1951)
55. Ketchel, M. M., and Williams, C. M., *Biol. Bull.,* **109,** 64–74 (1955)
56. King, R. C., and Burnett, R. G., *Science,* **126,** 562 (1957)
57. Kohring, V., *J. Morphol.,* **49,** 45–137 (1930)
58. Krause, G., *Biol. Zentr.,* **59,** 495–536 (1939)
59. Kuroda, Y., *Zool. Mag. (Tokyo),* **63,** 75, (1954)
60. Kuroda, Y., *Idengaku Zasshi,* **29,** 163 (1954)
61. Kuroda, Y., and Tamura, S., *Zool. Mag. (Tokyo),* **64,** 384 (1955)
62. Kuroda, Y., and Tamura, S., *Zool. Mag. (Tokyo),* **65,** 222 (1956)
63. Kuroda, Y., and Tamura, S., *Zool. Mag. (Tokyo),* **65,** 304–5 (1956)
64. Kuroda, Y., and Tamura, S., *Zool. Mag. (Tokyo),* **66,** 10 (1957)
65. Kuroda, Y., and Tamura, S., *Med. J. Osaka Univ.,* **7,** 137–44 (1956)
66. Kuroda, Y., and Yamaguchi, K., *Idengaku Zasshi,* **31,** 98–103 (1956)
67. Lazarencko, T., *Z. Mikroskop anat. Forsch.,* **3,** 409–99 (1925)
68. Lewis, M. R., *Anat. Record,* **10,** 287–99 (1916)
69. Lewis, M. R., and Robertson, W. R. B., *Biol. Bull.,* **30,** 99–124 (1916)
69a. Loeb, M. J., *The Culture of Insect Tissues* (Master's thesis, Cornell Univ., Ithaca, N.Y., 94 pp., 1957)
70. Loeb, M. J., and Schneiderman, H. A., *Ann. Entomol. Soc. Am.,* **49,** 493–94 (1956)
71. Ludwig, D., Tracey, K. M., and Burns, M. L., *Ann. Entomol. Soc. Am.,* **50,** 244–46 (1957)
72. Lüscher, M., *Nature,* **160,** 873–74 (1947)
73. Lüscher, M., *Rev. suisse zool.,* **55,** 227–32 (1948)
74. Maramorosch, K., *Virology,* **2,** 369–76 (1956)
75. Martignoni, M. E., Zitcer, E. M., and Wagner, R. P., *Science,* **128,** 360 (1958)
76. Millara, P., *Compt. rend. soc. biol.,* **140,** 1006–8 (1946)
76a. Morgan, J. F., *Bacteriol. Rev.,* **22,** 20–45 (1958)
77. Morgan, J. F., Morton, H. J., and Parker, R. C., *Proc. Soc. Expl. Biol. Med.,* **73,** 1–8 (1950)
78. Murray, M. R., *Biol. Bull.,* **50,** 210–35 (1926)

79. Murray, M. R., and Kopech, G., *Bibliography of the Research in Tissue Culture* (Academic Press, Inc., New York, N.Y., 2 vols., 1741 pp., 1953)
80. Nauk, E. G., and Weyer, F., *Zentr. Bakteriol Abt. 1. Orig.*, **147**, 365–76 (1941)
81. Palliot, A., *Bull. histol. appl. physiol. et pathol. et tech. micr.*, **1**, 216–223 (1924)
82. Parker, R. C., *Methods of Tissue Culture*, 2nd ed. (Paul B. Hoeber, Inc., New York, N.Y., 1950)
83. Pfeiffer, H. H., *Arch. exptl. Zellforsch. Gewebezücht.*, **20**, 225–29 (1937)
84. Pfeiffer, H. H., *Naturwissenschaften*, **27**, 389–90 (1939)
85. Pfeiffer, H. H., *Arch. exptl. Zellforsch. Gewebezücht.*, **24**, 273–87 (1942)
86. Pfeiffer, H. H., *Naturwissenschaften*, **31**, 47–48 (1943)
87. Pomerat, C. M., *Methods in Med. Research*, **4**, 198–291 (1951)
88. Pomerat, C. M., and Leake, C. D., *Ann. N.Y. Acad. Sci.*, **58**, 1110–124 (1954)
89. Price, W. H., *Public Health Repts. (U.S.)*, **71**, 125–38 (1956)
90. Price, W. H. (Personal communication, 1957)
91. Puck, T. T., and Marcus, P. I., *Proc. Natl. Acad. Sci. U.S.*, **41**, 432–37 (1955)
92. Ragab, H. A., *Trans. Roy. Soc. Trop. Med. Hyg.*, **43**, 225–30 (1949)
93. Ris, H., *Biol. Bull.*, **96**, 90–106 (1949)
94. Ross, J. D., and Syverton, J. T., *Ann. Rev. Microbiol.*, **11**, 459–508 (1957)
95. Sanford, K. K., *Methods in Med. Research*, **4**, 219–21 (1951)
96. Sanford, K. K., Earle, W. R., and Likely, G. D., *J. Natl. Cancer Inst.*, **9**, 229–46 (1948)
97. Sang., J. H., *J. Exptl. Biol.*, **33**, 45–72 (1956)
97a. Schmidt, E. L., and Williams, C. M., *Anat. Record*, **105**, 487 (1949)
98. Schmidt, E. L., and Williams, C. M., *Biol. Bull.*, **105**, 174–87 (1953)
99. Schmidtmann, M., *Z. ges. exptl. Med.*, **45**, 714–42 (1925)
100. Schneiderman, H. A. (Personal communication, 1957)
101. Schneiderman, H. A., Ketchel, M. M., and Williams, C. M., *Biol. Bull.*, **105**, 188–99 (1953)
102. Shaw, E. I., *Exptl. Cell Research*, **9**, 489–501 (1955)
103. Shaw, E. I., *Exptl. Cell Research*, **11**, 580–86 (1956)
104. St. Amand, G. S., and Tipton, S. R., *Science*, **119**, 93–94 (1954)
105. Stern, C., *Growth*, **4**, 377–82 (1940)
106. Takakusa, S., *Z. Zellforsch. mikroskop. Anat.*, **1**, 22–28 (1924)
107. Takeda, K., *Kyoto-ikadaigakugasshi*, **21**, 905–6 (1937)
108. Taylor, A., *Ann. Entomol. Soc. Am.*, **28**, 135–48 (1935)
109. Tobias, J. M., *J. Cellular Comp. Physiol.*, **31**, 143–48 (1948)
110. Trager, W., *J. Exptl. Med.*, **61**, 501–13 (1935)
111. Trager, W., *J. Parasitol.*, **23**, 226–27 (1937)
112. Trager, W., *Am. J. Trop. Med.*, **18**, 387–93 (1938)
113. Trager, W., in *Insect Physiology*, Chap. 14 (K. Roeder, Ed., John Wiley & Sons, Inc., New York, N.Y., 1953)
114. Vago, C., and Chastang, S., *Experientia*, **14**, 110-11 (1958)
115. Waymouth, C., *Methods in Med. Research*, **4**, 225–32 (1951)
116. Waymouth, C., *Intern. Rev. Cytol.*, **3**, 1–68 (1954)
117. Wermel, E. M., *Byull. Eksptl. Biol. Med.*, **5**, 6–9 (1938)
118. Weyer, F., *Z. Bakt. Parasitol. Infekt. Hyg.*, **159**, 13–22 (1952)

119. White, P. R., *The Cultivation of Animal and Plant Cells* (The Ronald Press Company, New York, N.Y., 239 pp., 1954)
120. Willmer, E. N., *Tissue Culture,* 2nd ed. (Methuen & Co., Ltd., London, England, 175 pp., 1954)
121. Wyatt, G. R., Loughheed, T. C., and Wyatt, S. S., *J. Gen. Physiol.,* **39,** 853–68 (1956)
122. Wyatt, G. R., and Kalf, G. F., *J. Gen. Physiol.,* **40,** 833–47 (1957)
123. Wyatt, S. S., *J. Gen. Physiol.,* **39,** 841–52 (1956)

PHEROMONES (ECTOHORMONES) IN INSECTS[1]

By Peter Karlson and Adolf Butenandt[2]
Max-Planck-Institut für Biochemie, Munich, Germany

DEFINITIONS

During the last few decades numerous substances have been investigated that resemble hormones in some respects but actually cannot be called hormones. The attractant of a moth, to cite an example, is produced and secreted by certain glands just as is a hormone; even the minutest amounts cause a reaction in the receptor organ (antenna of male) which induces the male to copulate. But, contrary to hormones, this substance is released to the outside, and not into the blood. It does not serve the humoral correlation inside the organism, but rather acts among individuals. Bethe (8) called such substances "ectohormones," and some authors have followed him. If, however, hormones are defined as products of incretory glands, then the word ectohormone (= ectoincretion) constitutes a contradiction in itself. We feel that the concept of hormone ought not be stretched too far; it is more convenient to invent a new concept.

Having consulted a few colleagues with experience in the same field, we should like to propose to name such substances "pheromones." The word is derived from the Greek *pherein* (to carry) and *horman* (to exite, to stimulate). "Pheromone" should designate substances that are secreted by an animal to the outside and cause a specific reaction in a receiving individual of the same species, e.g., a release of certain behavior or a determination of physiologic development. Pheromones, messengers among individuals, will then be on the same level as hormones, gamones (fertilizing substances), and termones (determining substances).

Pheromonal secretion need not occur in definite glands (though that is the case normally); a cuticular excretion may also be a pheromone. The method of acceptance in the receptor individual may likewise vary, i.e., it may be olfactory or oral. Olfactorily acting pheromones [e.g., the sex attractant of *Bombyx mori* (Linnaeus)] are received by the chemoreceptors of the antennae. Thence a nervous stimulus passes to the central nervous system and causes a certain behavior pattern. Some orally active pheromones may stimulate the chemoreceptors of the sense of taste in a similar manner.

The terminology of behavioral investigators would call pheromones "chemical releasers," in principle indistinguishable from optical or acoustical releasers. Yet physiological and biochemical aspects motivate us to prefer the definition given above in order to exclude other chemical stimu-

[1] The survey of the literature pertaining to this review was completed in May, 1958.

[2] Translator: Charles Doering, Stanford University, Stanford, California.

lants from the class of pheromones, especially food attractants, blossom fragrances, and insect repellents. The latter substances, although chemical releasers, do not belong to pheromones because they are not produced by individuals of the same species. Beyond that, strict species specificity is not demanded for the effect of pheromones; some overlapping is likely to occur with closely related species.

According to our definition, pheromones function as chemical messengers among individuals. Therefore, they presuppose social behavior, and it is not astonishing at all to find numerous examples of the occasionally called "social hormones" among the social insects.

It should be mentioned, that sex attractants are found among crabs, and even warning substances (*Schreckstoffe*) of fishes are pheromones, too. The same can be said of "territorial demarkation substances" of carnivorous animals. But the latter have been investigated very little.

This review will limit itself to pheromones of insects. First it will treat the sex attractants and marking scents as olfactorily active pheromones, then the queen substance of bees and active principles of sexual termites as orally active pheromones. Finally—with some reservations—it will discuss the determining substance in royal jelly.

OLFACTORILY ACTING PHEROMONES

The Sex Pheromones (Sex Attractants)

The sex attractants of insects are the most thoroughly investigated pheromones. This results in part from considerable commercial importance connected with the possible use of attractants for control of pest insects. Field tests with attractant traps have yielded some promising results, at least in limited areas. It is remarkable that such pheromones are not found exclusively among social insects, but also in various other insect orders. Hence, they will be treated according to the insect orders, with the Lepidoptera first, because they have been studied best.

Lepidoptera.—Sex attractants are known among various species [for reviews see Butenandt (14); Götz (40)]. The pheromone of the moth of the silkworm, *B. mori,* has been studied most thoroughly up to now; it was obtained in a nearly pure state (as the *p*-nitroazobenzenecarboxylic ester) by Butenandt *et al.* (13, 14, 50).

The sex attractant is formed in the lateral glands (*sacculi laterales*) of the last abdominal segment. The female is able to protrude and retract these glands, and in this manner she regulates the release of the attractant. Fertilized females are said to produce hardly any pheromone. Consequently, young virgin females of *B. mori* were raised for the collection of the attractant. The last segments containing the *sacculi laterales* were detached and conserved in petroleum ether.

The enrichment procedure was followed by bioassay based on the behavior of *B. mori* males when confronted with the attractant. They react

by becoming generally excited, beating their wings, and dancing erratically; at high concentration they attempt to copulate with the odoriferous object.

For assay the substance is dissolved in petroleum ether, a glass rod is wet with this solution and brought within ½ to 1 cm. of the antennae of a male. The reaction of the male is observed. Every concentration is evaluated on 20 animals. One sex attractant unit is reached when at least 10 animals have reacted postively; it is expressed in μg./ml. solution. Such a behavior test is simple to execute, but not very satisfactory for quantitative determinations. Concentration differences of only 1:10 may be distinguished with some certainty.

The scent glands are collected and extracted with petroleum ether. The extract is hydrolyzed with alkali, and the neutral fractions are worked up further (12). Alcohols are separated out as their succinic monoesters; this alcohol fraction proved to be active. Since the free pheromone is rather volatile, the *p*-nitroazobenzenecarboxylic esters of the alcohols in the mixture were prepared. The esters are physiologically inactive, but hydrolysis liberates the active substance which can be assayed.

Chromatography on Al_2O_3 combined with repeated Craig distribution (counter-current distribution) netted considerable purification. Distribution chromatography removed more impurities and yielded indications as to the molecular weight of the active principle. In addition to bioassays, paper chromatography was employed to check the purity. At last paper and distribution chromatographical purity was achieved. The hydrolysis product of such a preparation possessed one sex attractant unit with less than 10^{-5} μg./ml. The actual yield, however, was extremely low: only 5.3 mg. of the derivative was obtained from glands of 313,000 female silk moths.

It is suspected that the *Bombyx* attractant is a poly-unsaturated primary alcohol; it probably contains more than 10 carbon atoms. These properties were concluded from UV-absorption and chromatographical data. However, a thorough analysis of the natural product is yet to be performed.

Interesting enough, the physiologically inactive fatty acid removed during the purification of the sex pheromone become active alcohols with attractant properties after reduction by lithium aluminium hydride (37). One of the carboxylic acids in the mixture may be closely related structurally to the attractant and can be reduced to the latter. On the other hand, substances acting like the *Bombyx* pheromone at high concentrations are known; besides cyclohexanone and cycloheptanone, they are mainly unsaturated primary alcohols (the sex attractant units are given in parentheses): Δ^2-hexenol (4 mg./ml.), $\Delta^{2,4}$-hexadienol (0.1 mg./ml.), and $\Delta^{2,4,6}$-octatrienol (0.001 mg./ml.). The most potent compound of this series is 10^6 times weaker than the natural substance. In addition, these compounds stimulate even *B. mori* females to beating of wings, in contrast to the natural sex attractant (83, 84). They also give a different electroantennogram (see later). All four stereoisomers of $\Delta^{2,4}$-hexadienol (15a) show the same activity, indicating that these synthetic compounds are

rather unspecific stimulants with some resemblance to the food attractants or insect repellents.

Independently of Butenandt & Hecker (14, 50), the fractionation of *Bombyx* extracts and purification of the pheromone was also reported on by Makino *et al.* (71). However, their product was not characterized as uniform; if the bioassays are comparable (the method of testing differed), then Makino's "bombixin" is about 100 times weaker. Probably it is a concentrate containing the pheromone along with several other inactive admixtures.

Another report, by Amin (5), must be rejected as completely wrong (15). No proof whatsoever is indicated for the biological activity of his isolated product, his identification of dimethylamine (as *p*-nitroazobenzenecarboxylic amide) has no significance for the chemistry of the *Bombyx* pheromone. Dimethylamine was bioassayed in our laboratory; it becomes active only at 10,000 μg./ml., while the best preparations of *Bombyx* are active already at 10^{-6} to 10^{-5} μg./ml. No further comment is necessary.

The *Bombyx* pheromone is perceived by olfactory receptors of the antennae. The morphology of the *Bombyx* antennae was reinvestigated in this connection by Schneider & Kaissling (85, 86). The very numerous sensilla may be classified into 15 main groups; chemoreception may be assigned tentatively to sensilla basiconica or sensilla coeloconica (83) or both. With electrophysiological methods, Schneider (82, 83, 84) succeeded in recording action potentials from the antennae. On stimulation with natural *Bombyx* sex attractant, a characteristic response appeared in the electroantennogram, while other olfactory stimuli or narcotica resulted in different electroantennograms. The electrophysiological test is capable of distinguishing qualitatively between substances such as sorbinol, cycloheptanone, and others on the one hand and pheromone on the other. The behavior test recognizes only great differences in threshold of stimulus, i.e., the sex attractant unit. Furthermore, doses 1000 times smaller still give a reaction in the electrophysiological test. As shown by a rough approximation, a few 100 molecules per receptor suffice for the reaction.

Behavior-physiological analysis of the sex attractant shows it to be the excitant [Schneider (83); Schwink (88, 89); Sengün (91)]. It indicates the proximity of females. The attraction as such relies on the sense of air current; the excited females orient themselves against the wind direction. Without pheromone stimulation, air currents do not aid in orienting the insects. Concentration gradients of pheromone are unimportant for behavior physiology. The same principle operates for moths in free flight [experiments on *Porthetria dispar* (Linnaeus) (89)] and explains biological observations of the attraction. Such a rheotactic orientation may be operative across vast distances. On the contrary, the supposition merely of a concentration-dependent olfaction has always made it difficult to understand directional flight approach.

The sex pheromone of the gypsy moth, *Porthetria dispar* (Linnaeus)

[=*Lymantria dispar* (Linnaeus)] was investigated diligently by Acree and co-workers (1, 2, 47). It may be extracted with benzene from the abdominal tips; the active principle is found in the unsaponifiable fraction of the extract and is an alcohol, as was shown by ester formation with phthalic and azobenzenecarboxylic acid. Chromatography also afforded a method of appreciable purification and hinted at the possibility of there being several active substances in the crude extract, possibly two different alcohols along with their esters. UV-absorption at 230 mμ and 285 mμ points toward double bonds; hydrogenation increased activity in some experiments (47), but this observation could not be reproduced later (H. L. Haller, private communication). Work was complicated by the difficulty of bioassays, namely field experiments with attractivity in infested areas. Perhaps decisive advances will be possible only after introduction of electrophysiological methods of assay.

An attempt to obtain and isolate the sex pheromone of *Euproctis chrysorrhoea* (Linnaeus) by condensation of air streams failed (Inhoffen, 51).

The attractant of the cotton pest *Prodenia litura* (Fabricius) was studied by Flaschenträger & Amin (30). It is a volatile, lipide-soluble substance, possibly an alcohol. Again difficulties arose in the application of bioassays. Flaschenträger, Amin, & Jarczik (31) reported an assay in an "odour analyzer" which now proves to be irreproducible (Jarczik, private communication).

Olfactory stimuli affect the butterfly *Argynnis paphia* (Linnaeus) only across short distances; for the release of copulation instincts optomotory stimuli are responsible (70).

Barth's reports (7) on sex odors of pyralids were corrected to some extent by Schwinck (87). Especially Barth's claim that the scent of *Plodia* is unstable was not corroborated by her. When test males were placed into dishes 66 hr. after removal of females they still reacted vehemently with whirling dances. Thus, the rather peculiar hypotheses that the energy freed during decay of the scent is responsible for its far-reaching attractiveness may all be discarded finally. The attractant of the pyralids does not seem to be strictly species specific. During behavior experiments, Schwinck found no difference of reaction of *Plodia* or *Ephestia* males toward pheromones of the same or of the other species. An exact determination of the relative stimuli thresholds will be possible only with isolated and enriched pheromone preparations (cf. 7, 40, 89, 90).

The release of sex scents is not constant throughout the day. For *Polychrosis botrana* (Schiffermüller), Götz (38, 39) observed a maximum in the early morning hours. He even reported on a large-scale experiment in an encaged area of 0.12 acres with practical application for the extermination of pests. Sex pheromones of the majority of the harmful insects unfortunately are not yet available for field testing. Trapping trials using ♀ ♀ as bait would seem to be unreliable in some respects.

Coleoptera.—Both the male and the female beetles of the mealworm,

Tenebrio molitor Linnaeus produce specific sex pheromones [Valentine (97)]. The production site is found in glands on the last abdominal segment. The female odor is much more potent for males than the male odor for the females, as was found by experiments with scent-carrying glass rods. Attempts at isolation are not reported.

Female June beetles, *Phyllophaga lancelota* Say, are as strongly attracted by isoamylamine as by the sex pheromone [cited from Dethier (28)].

Hymenoptera.—The sex pheromones of some bumble bee species were investigated by Kullenberg (57). They are excreted by the abdomen. Scent-bearing dummies attract males, which may even attempt copulation. The odorous substance was not isolated, but the dummies were rubbed mechanically with freshly caught females and loaded with the attractant in this way. In addition, dummies carrying various other odors (farnesol, citronellol, citronellal, etc.) were set up in field experiments to study the approach of *Macropis labiata* (Fabricius), *Crabro cribarius* (Linnaeus), and *Gorytes campestris* (Linnaeus). Preferred were farnesol, citronellal, and hydroxycitronellal, also the fragrance of *Ophrys insectifera* Linnaeus blossoms. These substances smell very similar to the bumble bee pheromone, even to the human nose. It is difficult to judge the importance of these results, e.g., the attraction of *M. labiata* by farnesol. Neither the concentration of the test substance nor that of the natural pheromone is known. It is quite possible, therefore, that it is an unspecific effect, as with sorbinol on *Bombyx,* or an effect of marking scents (see later).

Kullenberg examined the scents of 49 Hymenoptera species and indicated substances or mixtures with comparable odor for all of them. Even Kullenberg leaves open the question as to how far the human nose can serve to differentiate and identify these odors; this may be the weakest point in his careful investigation. But it should not be overlooked that the necessary chemical characterization of the scents is not possible yet: neither can sufficient material be gathered nor a usable assay found to enable the purification by chemical methods.

Orthoptera.—Roth & Willis found that female cockroaches [*Periplaneta americana* (Linnaeus)] produce a specific sex attractant (81). It adheres to the support, e.g., filter paper, on which the females may sit, and it may be extracted from it by petroleum ether. By stirring their antennae, spreading their wings, and attempting copulation, males react toward this pheromone. Wharton *et al.* developed a quantitative assay (109, 110) utilizing direct comparison with a standardized solution that permits the determination of concentrations to an accuracy of ± 40 per cent. This assay is probably the most exact described for pheromones. Experiments for chemical purification of the active substance are being carried on.

Generally, the pheromone is released only by adult *P. americana* females; pheromone-producing nymphs are an exception [but compare *Blattella germanica* (Linnaeus) (81)]. After copulation, pheromone production de-

creases swiftly; it decreases slowly when oothecae are produced parthenogenetically by virgin females. Irradiation with cathode rays (1000 to 2500 r) impedes pheromone production at first, but during recovery period it rises beyond the normal value (111).

For further scents of unknown biological significance produced by cockroaches see (81b).

Hemiptera: Heteroptera.—Males of the tropical water bug *Lethocerus indicus* (Lepeletier & Serville) (=*Belostoma indica*), produce a scent that may possibly belong to the group of sex pheromones. This tentative classification is based only on the fact of sex-specific occurrence; unfortunately no biological observations on the functions of that substance are available. It is formed in a couple of white tubules, 4 cm. long and 2 to 3 mm. thick, which are arranged dorsally in the abdomen. Approximately 0.02 ml. of a clear fluid with cinnamonlike odor may be removed from these per animal. This extract is used by the natives of Southeast Asia as a spice for greasy foods.

Chemical studies of the substance by Butenandt & Tam (17) proved it to be a carboxylic ester with the formula $C_6H_{11}O \cdot CO \cdot CH_3$. Oxidative degradation yielded acetic and butyric acid; the latter must have come from the alcohol part of the ester. Therefore, the *B. indica* scent must possess the constitution of Δ^2-hexenol acetate: $CH_3-CH_2-CH_2-CH=CH-CH_2O-CO-CH_3$.

Synthetic hexenol acetate was identical in all respects with the natural product. If it should turn out to be a sex attractant, then it would be the first pheromone whose chemical constitution is known and which is synthetically available.

In this context it may interest that a compound of very similar chemical constitution was found in both sexes of the cockroach, *Eurycotis floridana* (Walker), (81a). It is Δ^2-hexenal, $CH_3-CH_2-CH_2-CH=CH-CHO$, and serves the cockroach as a repellent or defensive substance.

Marking Scents

Apis mellifera Linnaeus.—Honey bees are attracted by members of the same species not necessarily of the same hive. Lecomte (58) as well as Free & Butler (33) were able to demonstrate that olfactory stimuli are sufficient to attract worker bees (just as vibratory stimuli alone suffice) and that the scent is produced in Nassanoff's glands. When such glands were excised and placed in a cage, they were frequented preferentially. In experiments with an olfactometer, individual bees had to choose between a scented and scentfree air current. The scented path was traveled about 4 to 5 times more often. With this arrangement it was also shown that the scent is not specific for each hive. The olfactometer experiment should be usable as a bioassay method to follow the purification of the extracted scent and thus isolate the pure substance. Also Free & Butler's "cage experiment" may be adaptable for bioassay.

Similar results had already been obtained earlier by von Frisch & Rösch (34). In their training experiments, however, a hive-specific component was superimposed on the Nassanoff pheromone. Renner (*76, 77*) repeated these experiments with improved methods. The Nassanoff pheromone was absorbed by filter-paper strips and thus obtained free of any interfering body odors. Both the training and the choice experiments revealed the pheromone to be colony nonspecific.

According to experiments by Kalmus & Ribbands [(55; cf. also (22)], colony-specific scents arise from preferred foods; one section of a beehive developed its own peculiar odor after feeding on certain very fragrant types of honey. On the other hand, some hives became scent-identical in a region where only one source of food was available.

Lecomte (59) was also able to distinguish between species-specific and hive-specific scents in his work with feeding techniques. Along with the pheromone of Nassanoff's glands, he discovered a generally repelling material comparable to repellents of fishes and two hive-specific odorous substances of different effects. Whether they are well-defined scents or simply repulsive odors becoming conspicuous in this choice experiment remains to be shown. Feeding technique alone probably will not be able to answer this question satisfactorily.

Other Hymenoptera.—In their routine flights bumble bees orientate themselves by self-laid scent deposits. The secretion of the mandibular gland [Haas (46)] is used to mark blossoms, leaves, and spots near the nest; henceforth, the bees pass over these "signposts" again and again. The scent of *Bombus hortorum* (Linnaeus) approximates the fragrance of linden trees (57). Hydroxycitronellal imitates it so well that bumble bees can no longer distinguish it from the true scent. The marking scent has attractive but not exciting properties and affects both sexes. [For olfactory analyses of scents from cephalic glands of other aculeate Hymenoptera, see Kullenberg (57).]

It is noteworthy that in bumble bees abdominal organs release the sex attractant and mandibular glands the marking scent, whereas the marking substance of *Apis mellifera* is released by the abdominal gland and the mandibular gland may be responsible for the "queen substance." Here there appears to be a variation in the importance of pheromones which adapts the individual for colony life.

Tracking substances exist among ants that mark their well-known roads. *Formica rufa* Linnaeus seems to use formic acid for this purpose. Other ants have special scent materials which have so far been studied very little. Secretions of the cephalic gland of *Atta sexdens rubripilosa* Forel appear to act as a warning substance, according to M. Lindauer (unpublished observation). The substance is a mixture, similar to vegetable essential oils, containing chiefly citral. It was identified as dinitrophenylhydrazone (B. Linzen, unpublished data).

ORALLY ACTING PHEROMONES

Queen Substance of the Honey Bee

Soon after removal of the queen from a colony of honey bees (*Apis mellifera*), conspicuous changes in the behavior of the worker bees may be observed. Besides general restlessness on the part of the bees (they may even attach themselves to another colony), cells containing young larvae are changed into emergency queen cells (18, 73). Furthermore, the ovaries of worker bees normally atrophied begin to develop.

Proof of pheromone production.—Three different groups of investigators have studied the mechanism of this behavior: Chauvin & Pain (25, 75), de Groot & Voogd (45, 104, 105), and Butler and his co-workers (18 to 24). They agree that a substance is distributed by the queen to the worker bees. The transfer of the substance (queen pheromone) informs the workers of the presence of the queen, retards the development of their ovaries, and influences their behavior. These effects were demonstrated experimentally in several ways: (*a*) The worker bees must have contact with their queen; if the queen is separated from them by a double wire screen, the growth of ovaries is no longer inhibited. (*b*) Unfertilized and even dead queens possess the same influence as fertilized and laying queens. However, if the dead queen is extracted with alcohol or acetone, she loses her effectiveness. (*c*) The extract contains the active principle. When the dead queen is rubbed with it and reinserted into the colony, ovary development is again impeded.

Voogd (105) believes that the queen pheromone must be offered in a particular manner, i.e., either on the body of a dead bee or on some other object. [Pain (75) uses elder pith, Voogd (104) wood splints.] But Butler & Gibbons obtained good results by feeding the extract (mixed with syrup or drinking water), although the effect was weaker. Either method precludes the exact measurement of administered material. Butler probably is correct in assuming that the amount of ingested material is critical (maybe its concentration, too?). Both amount and concentration are probably less in feeding solutions. A psychic stimulation, as supposed by Voogd, seems to be rather improbable, since the offering is just as effective on inanimate materials as on a dead queen.

The chemical nature of queen substance can only be guessed at presently. Pain (75) observed an UV-absorption at 248mμ in the queen extract which was absent in the worker extract. It is yet uncertain whether it may be ascribed to the pheromone. From solubilities in alcohol, acetone, and chloroform, Pain concludes that it is a steroid or a wax; Voogd concurs that it should be a fatty acid. Butler, too, favors a relationship to steroids (22). But definite statements will be possible only after the isolation of the pure pheromone. It should be noted that the ovary-inhibiting hormone of crustaceans may replace queen substance in its effect and vice versa [Carlisle & Butler (24)].

Mechanism of pheromone action.—The question arose (104, 105) whether queen pheromone affects ovaries directly or whether it comprises only a link in a chain of factors that cause behavioral differences and inhibit the development of ovaries. Carlisle & Butler's observation, cited above, seems to support the first possibility, namely that the pheromone acts directly, very much like a hormone. On the other hand, Butler found that worker bees pass along queen substance with the content of their honey stomachs and thus dilute the substance considerably. Still the amount produced by the queen is sufficient to supply tens of thousands of workers. All this would be more intelligible if one could assume that the substance is received gustatively and then acts via the central nervous system. The brain would influence the behavior directly, the ovarial development indirectly, i.e., endocrinously (perhaps by neurosecretion). In this connection it would be interesting to know whether injection of queen pheromone has the same effect as feeding.

Ovary-inhibiting factors have been observed among other insects (29). It would be worth while to determine whether extracts with the same activity as the queen pheromone could be obtained from other insects. If they could be obtained, we would have good indication that some active substances assume novel roles in social insects. Perhaps there is a connection with the marking substances of other Hymenoptera (see Page 46). According to Haas, these substances are produced in mandibular glands (46). It is assumed that queen pheromone is formed in head glands and distributed over the body, and consequently is found in greater concentration on the abdomen and head.

Queen Pheromone of Ants

Ants seem to possess a substance with functions very closely related to those of queen substance of bees. Three typical behavior patterns of worker ants are evoked by it [Stumper (92 to 95)]: (*a*) crowding of the queen, often by droves of attracted workers; (*b*) intensive licking of the queen (this has been interpreted as body care, but which is more likely an intake of the queen substance by the workers); (*c*) transportation of the queen, especially after disturbance of the nest.

Stumper succeeded in showing that a dead queen serves these functions just as well as a live one; in fact, even a piece of some inorganic material impregnated with queen extract has the same effect. These phenomena are particularly apparent with *Lasius alienus* (Foerster) and *Pheidole pallidula* (Nylander). Petroleum ether is more suitable than diethyl ether or ethanol for extraction of queens. Only repeated extraction will remove the queen substance entirely. For bioassay of the active material, the attractivity of an object (elder pith) impregnated with extract may be employed. It is easy to observe and permits even semiquantitative evaluation. The activity decreases and disappears after a few days, either by evaporation or oxidation, or because of licking by worker ants.

Lasius ☿ bodies impregnated with extracts of *Pheidole* queens are ac-

cepted as *Pheidole* queens, but are destroyed after one day. The reverse experiment is rather unsuccessful, which demonstrates the specificity of the material. The amounts of *Pheidole* queen substance obtained hitherto are minute, and no purification has been achieved yet. Stumper proposes a probable relationship to lipides of the epicuticule, at least to the extent that pheromones are dissolved in the wax layer.

Earlier observations by Bier [(9, 10); detailed discussion in (11)] on the fertility of workers of *Leptothorax tuberum unifasciatus* (Latreille) and *Formica rufa pratensis* Linnaeus showed that functional queens inhibit ovary maturation of worker ants. Bier proved that (*a*) the odor of females is not responsible for this effect, and (*b*) the inhibiting effect originating with the queen is spread by the workers [probably by feeding; cf. Lange (57a)]. The analogy to the queen pheromone of bees is complete.

In our opinion, the majority of observations may be explained by supposing an inhibition of the ovary development by some substance of ♀ ants. Maybe it is perceived through chemoreception and functions as a releaser, since there is a close relationship between behavior and ovary growth of workers [D. Otto, cited in (11)]. But Bier discusses a hypothesis that attributes "profertility" to food produced in the pharyngeal glands. According to it, workers of a normal nest relinquish this food to the queen and the larvae; only in their absence will workers consume the food themselves, develop ovaries, and eventually ovipost. These hypothetical profertile substances [postulated for bees also by Pain (74)] are most likely analogous to the active principle in royal jelly (see later). But perhaps they are to be classified as vitamins instead of pheromones. Further experiments, preferably with purified or even isolated preparations, will decide that question.

Pheromones of Termites

Termites, too, are known to employ substances produced by the caste of reproductives to influence the colony's members. Changed social structures, however, necessitate a changed pheromonal system.

As the first major difference, the termite colony possesses both male and female repoductives (king and queen). Second, the developmental status of workers differs: commonly they belong to juvenile instars and as "pseudergates" are capable of metamorphosis by means of additional molts into winged reproductives, into wingless supplementary reproductives, or into soldiers. The direction of changes is governed by the need of the colony. Grassi & Sandias (44a) must be credited for clarifying the relation between castes and social regulation. Later Grassé *et al.* (43, 44), Light (60), and Lüscher (62, 63, 64) expanded our knowledge greatly [for review see (11)].

Proof of pheromone production.—When the king and queen are removed from a termite colony, supplementary reproductives arise in very short time —within six days at the earliest with the thoroughly studied species *Kalotermes flavicollis* (Fabricius). They develop out of pseudergates by molting and are to be considered as true adult castes [Lüscher (62)]. To inhibit the development of supplementary reproductives is an important duty

of the primary reproductives. That this duty is carried out pheromonally has been shown by screen experiments. For example, following separation of a normal and an orphan colony by a screen, the latter will produce supplementary reproductives which, however, are soon eliminated, because antennal contact with their neighbors keeps the colony from "feeling" orphaned. Likewise, if a ♀ is attached to a screen in such a manner that her head belongs to one colony and her abdomen to the other, then she causes complete inhibition of the development of supplementary reproductives only in that colony in possession of her abdomen (plus a functional ♂). Therefore, the inhibitory substance is secreted by the abdomen and probably by the intestine.

Light was able to impede the production of supplementary reproductives by feeding of extracts [on *Zootermopsis* (60); for interpretation see Lüscher (64)]. Head extracts were particularly effective. Probably the pheromones are formed in the head, eliminated through the intestines, and ingested by larvae with the excretion. The larvae pass along this active substance to their colony members (probably by the same route). It can pass through a larva fixed to a screen and inhibit the production of supplementary reproductives in the orphan colony [Lüscher (65)].

The male pheromone alone is completely inactive; the female pheromone alone is feebly inhibitory for the generation of supplementary females. Inhibition occurs fully only when there is both a ♂ and ♀ reproductive present. Obviously, male and female pheromone must be administered together and at the same time.

Mechanism of termite pheromone action.—The molt to a supplementary reproductive that arises through lack of pheromone is an induced one; quite manifestly it starts before the normal molt of pseudergates. Recently molted larvae have a great competence for it, which decreases gradually and finally is lost completely as soon as the next pseudergate molt is determined [Lüscher (62, 64)]. Since molting is ordinarily induced by the endocrine system [neurosecretory cells, prothoracic glands, corpora allata; for review see Karlson (56)], the molt to supplementary reproductives was assumed to arise from premature prothoracic gland activity whose hormone (ecdysone, "growth and differentiation hormone") was supposed, in the absence of the corpus-allatum hormone, to lead to adult forms.

This last supposition was tested experimentally [Lüscher & Karlson (67)]. Injection or feeding of ecdysone (crystalline prothoracic gland hormone) induced molting of termites, as expected. But pseudergates resulted invariably and no adult forms (i.e., neither winged reproductives, nor supplementary reproductives, nor soldiers) even after application of large doses of the hormone. Hence, the deficiency of pheromone is responsible not only for the activation of prothoracic glands (which is realized in all likelihood by way of neurosecretory cells of the brain) but also for some other changes in the hormone balance.

According to histological studies by Lüscher (66a; cf. 53, 54), the

corpora allata begin to grow with the induction of the molt to supplementary reproductives. They attain a four- to fivefold volume six days later, at the time of ecdysis. This molt should, therefore, be the result of joint stimulation by prothoracic glands and corpora allata. Possibly additional factors enter the picture [an adult factor, Lüscher (66)], for when molting is elicited by administration of ecdysone simultaneously with implantation of corpora allata, a supplementary reproductive does not result. On the contrary, nymphs change to soldiers if the implantation occurs at least 15 days before molting [Lüscher, (66a)].

Although developmental physiology of the various castes is not yet completely clear, it is well established that pheromone deficiency causes a specialized form of molting via the endocrine system. Parallel to this is the initiation of development after diapause induced by a variety of stimuli and carried out by the endocrine system.

Finally, it should be emphasized that the lack of pheromone does not affect the behavior primarily, but rather represents a physiological differentiation. Herein lies the difference from the majority of examples presented up to now. In many experiments the behavior of termites, as expressed by "jittering" during confrontation and by eliminating supernumerary reproductives, runs counter the observed determination. This does not really preclude any effect through gustative chemoreception and the central nervous system, but makes it rather improbable.

The Determining Substance in Royal Jelly

Both castes of female bees, sterile workers and fertile queens, originate from identical eggs. Later, on the second or third day of the larval development, differentiation is initiated by means of the food given to the larvae. We can distinguish between worker food and queen food or "royal jelly" [for review of royal jelly, see Johansson (52)].

Opinions concerning the determining factor in this food are not in agreement. Numerous authors—foremost among them is Haydak (48)—presume that abundant rations of high-quality food are responsible for the development of queens and do not believe in the existence of a specific determining substance. The entire problem would not have to be treated here, if their view were substantiated. But in antithesis it is postulated that certain substances in queen food determine larvae to queens. Rhein (80) goes even further and discusses factors (*a*) determining larvae to worker bees, (*b*) predetermining and (*c*) determining them to queens. They are presumed to occur in the food of worker larvae, juvenile, and mature queen larvae, respectively.

By feeding royal jelly in *in vitro* experiments, Weaver (106, 107, 108) successfully raised a few pupae which later developed into queens or mixed forms between workers and queens. In the event that this method is improved for bioassay, foods and extracts thereof could be tested and a verdict be reached between those two hypotheses.

Even if a specific factor is discovered in royal jelly, it must still be decided whether or not it is a vitamin ingested by nurse bees and accumulated in the pharyngeal gland secretion. But it may also be produced by the bees themselves and fed to queen larvae exclusively. In the latter case only, the active principle could be termed "pheromone." In spite of these reservations, the present state of the problem shall be reviewed.

Chemistry of royal jelly.—There is no scarcity of attempts to resolve the composition of the food by chemical fractionation alone, without any bioassay [Chauvin (26); Haydak (48, 49); Melampy & Jones (72); Townsend & Lucas (96); cf. also (41)]. In addition to usual nutrients, surprising amounts of vitamins, primarily of the B group, are found in royal jelly. Pantothenic acid is detected in quantities up to 100 to 300 μg./gm. [but pantethein is lacking (H. Rembold & F. Lingens, unpublished data)]; biotin content is rather high, too. Food for royal juvenile larvae contains appreciably more biotin and pantothenic acid than that for royal mature larvae or worker larvae. These vitamins have been considered by some to be responsible for the determining effect, but this conclusion is highly questionable. A search for enzymes uncovered a low esterase activity, apparently nonspecific (6). By pharmacological methods no special effects could be recognized [Forster (32); cf. Chauvin (27)].

One acid makes up for a considerable portion of the lipide fraction (ca. 90 per cent). It was isolated by Townsend (96), and its constitution elucidated by Butenandt & Rembold (16) as ω-hydroxydecenoic acid:

$$HO—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—CH = CH—COOH$$

This acid may be responsible for the bactericidal and fungicidal properties of royal jelly. It has little significance in determination of larvae, in all probability, for it is found in food for worker bees as well as in that for queens (16).

Another substance occurring in royal jelly, but not in worker food, was isolated by Butenandt & Rembold (16a) and identified as biopterin, i.e., 2-amino-4-hydroxy-6-(L-erythro-1,2-dihydroxypropyl)-pteridine:

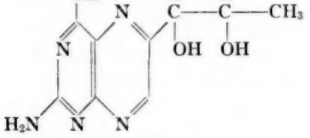


FIG. 1

This substance has been isolated from various sources (57a, 98, 103), including insects (32a, 99 to 102).

The amount of biopterin in food solutions is very small. A total of

500 gm. royal jelly yielded 4.7 mg. This compound was discovered in royal jelly by observing a weak fluorescence on paper chromatograms. The same method demonstrated the absence of biopterin in food for worker larvae. This result was verified microbiologically by means of biopterin-specific tests on *Crithidia fasciculata* Léger [(11a), H. Rembold & L. A. Wacker, unpublished data]. A comparative investigation of larvae of queens and workers indicated the same distinction. Whether or not biopterin is the suspected pheromone of royal jelly cannot be decided in absence of a bioassay method. The demonstration of qualitative differences in composition of the foods for both castes may support either the vitamin or pheromone hypothesis.

Mechanism of action.—Various hypotheses have been offered to explain how the food factor acts. Haydak (48) suspects that the deficient diet of worker larvae results in disturbances of the endocrine system. Especially the lack of ovarial hormones would influence secondary sex characteristics. But it should be kept in mind that true sex hormones have not yet been found in insects.

According to Rhein, the hormonal system also plays an important role (80). After feeding of nonphysiological nutrients or of compounded food, normal development is frequently disturbed and pupation does not set in. Rhein discusses the possible occurrence of ecdysone in royal jelly. In our own experiments, no activity was found by application of standard extraction procedure followed by *Calliphora* bioassay [sensitivity, 0.1 μg./gm. royal jelly (P. Karlson, unpublished data)]. Further, we are not able to confirm Altmann's observations (3, 4) of the effect of royal jelly on ovary development [G. Hanser & P. Karlson, unpublished data; cf. (56)]. In worker larvae Lukoschus (68, 69) noticed deviations of volume and histological appearance of the incretory glands which suggest hypofunction, especially of prothoracic glands. It is Lukoschus' assumption that worker larvae receive at the time of change of diet an inhibitory substance which lowers production of the prothoracotropic hormone in the brain and, in turn, the production of ecdysone. Therefore, he concludes, one should search for a "pheromone" in the food of worker larvae that could determine them to develop into worker bees by inhibiting ecdysone secretion. This argument is not compelling, however, for it is entirely possible that some other factor occurring in queen food is necessary for normal activity of hormonal glands. There is an analogy to several vitamins, whose lack leads to metamorphosis interference [folic acid deficiency in *Aedes aegypti* (Linnaeus) (42) and carnitine deficiency in *Tribolium* (35, 36); for review see Lipke & Fraenkel (61)].

CONCLUDING REMARKS

As already mentioned in the introduction (Page 39), the mechanism of olfactorily acting pheromones is clearly understood. They are highly ac-

tive stimulants which affect the central nervous system via the chemical sense. For orally acting pheromones, two possibilities are subject to discussion: (*a*) taste perception, completing the analogy to the group mentioned above, and (*b*) a biochemical mechanism, the substances being resorbed in the gastrointestinal tract, reaching the blood stream, and simulating hormones in their method of action. The alternative, "behavior *versus* developmental physiological determination," remains inconclusive with respect to the distinction given above. Sense stimulation may release a determination via the neurosecretory-endocrine system; hormones may effectuate a behavior pattern. In addition, lack of queen substances of bees causes a particular behavior (construction of emergency queen cells) as well as physiological development (growth of worker ovaries).

Indications concerning the mechanism of the orally acting substances are scarce and contradictory; they have been treated to some extent. A decision could be reached experimentally when the substances are injected. In the first case nothing would happen; in the second, the same effects would result as by oral uptake. Injections must necessarily be performed with purified materials, but they are not yet available.

Taking the different modes of action as a basis, a conceptual and definitive separation of the reviewed substances into two classes might be considered, i.e., one could term sensorily acting substances "telemones" and biochemically operating substances "pheromones." We regard it as premature to arrive at such a distinction. To isolate the discussed pheromones chemically pure and to gather more information about their mechanism seem of far greater importance.

LITERATURE CITED

1. Acree, F., Jr., *J. Econ. Entomol.*, **46**, 313–15 (1953)
2. Acree, F., Jr., *J. Econ. Entomol.*, **47**, 321–26 (1954)
3. Altmann, G., *Z. Bienenforsch.*, **1**, 124–27 (1952)
4. Altmann, G., *Z. Bienenforsch.*, **2**, 59–66 (1953)
5. Amin, El S., *J. Chem. Soc.*, 3764 (1957)
6. Ammon, R., and Zoch, E., *Arzneimittel-Forsch.*, **7**, 699–702 (1957)
7. Barth, R., *Zool. Jahrb. Abt. Allgem. Zool. Physiol. Tiere*, **58**, 297 (1937)
8. Bethe, A., *Naturwissenschaften*, **20**, 177–83 (1932)
9. Bier, K. H., *Ins. Soc.* **1**, 7–19 (1954)
10. Bier, K. H., *Ins. Soc.*, **3**, 177–84 (1956)
11. Bier, K. H., *Ergeb. Biol.*, **20**, 97–126 (1958)

11a. Broquist, H. P., and Albrecht, A. M., *Proc. Soc. Exptl. Biol. Med.*, **89**, 178–80, (1955)

12. Butenandt, A., *Jahrb. preuss Akad. Wiss.*, 97 (1939)
13. Butenandt, A., *Naturw. Rundschau*, **8**, 457–64 (1955)
14. Butenandt, A., *Nova Acta Leopoldina*, **17**, 445–71 (1955)
15. Butenandt, A., and Hecker, E., *J. Chem. Soc.*, 53 (1958)

15a. Butenandt, A., Hecker, E., and Zachau, H. G., *Chem. Ber.*, **88**, 1185–96 (1955)

16. Butenandt, A., and Rembold, H., *Z. physiol. Chem.*, **308**, 284–89 (1957)

16a. Butenandt, A., and Rembold, H., *Z. physiol. Chem.* (In press)

17. Butenandt, A., and Tam, N.-D., *Z. physiol. Chem.*, **308**, 277–83 (1957)
18. Butler, C. G., *Trans. Roy. Entomol. Soc (London)*, **105**, 11–29 (1954)
19. Butler, C. G., *Proc. Roy. Entomol. Soc. (London)*, [A]**31**, 12–16 (1956)
20. Butler, C. G., *Experientia*, **13**, 256–57 (1957)
21. Butler, C. G., *Ins. Soc.*, **4**, 211–23 (1957)
22. Butler, C. G., *Proc. Roy. Soc. (London)*, [B]**147**, 275–88 (1957)
23. Butler, C. G, and Gibbons, D. A., *J. Insect Physiol.*, **2**, 61–72 (1958)
24. Carlisle, D. B., and Butler, C. G., *Nature*, **177**, 276–77 (1956)
25. Chauvin, R., and Pain, J., *Experientia*, **12**, 354–55 (1956)
26. Chauvin, R., *Apiculteur*, (Sect. Sci.) Nov. 1956, 1–12
27. Chauvin, R., *Compt. rend.*, **243**, 1920–21 (1956)
28. Dethier, V. G., *Chemical Insect Attractants and Repellents* (The Blakiston Co., Philadelphia, Pa., 289 pp., 1947)
29. Engelmann, F., *J. Insect Physiol.*, **1**, 257–78 (1957)
30. Flaschenträger, B., and Amin, El S., *Nature*, **165**, 394 (1950)
31. Flaschenträger, B., Amin, El S., and Jarczyk, H. J., *Mikrochim. Acta*, 385–89 (1957)
32. Forster, W., *Über den Weiselfuttersaft und die Frage seiner Bedeutung für die Pharmakotherapie* (Doctoral thesis, Julian Maximilian University at Würzburg, Germany, 1956)

32a. Forrest, H. S., and Mitchell, H. K., *J. Am. Chem. Soc.*, **77**, 4865–69 (1955)

33. Free, J. B., and Butler, C. G., *Behaviour*, **7**, 304–16 (1955)
34. von Frisch, K., and Rösch, G. A., *Z. vergleich. Physiol.*, **4**, 1–21 (1926)
35. Fröbrich, G., *Naturwissenschaften*, **40**, 556 (1953)
36. Fröbrich, G., *Z. Vitamin-, Hormon- u. Fermentforsch.*, **6**, 1–24 (1954)
37. Gerok, W., *Die Säuren aus den Duftdrüsen des weiblichen Seidenspinners*

(Bombyx mori) *und ihre Beziehungen zum Sexuallockstoff* (Doctoral thesis, Eberhard Karls University at Tübingen, Germany, 1950)
38. Götz, B., *Z. angew. Entomol.*, **26,** 243–64 (1939)
39. Götz, B., *Wein u. Rebe*, **23,** 75–89 (1941)
40. Götz, B., *Experientia*, **7,** 408–18 (1951)
41. Goillot, C., *Compt. rend.*, **245,** 1082–84 (1957)
42. Goldberg, L., de Meillon, B., and Lavoipierre, M., *J. Exptl. Biol.*, **21,** 90–96 (1945)
43. Grassé, P. P., *Traité de Zoologie*, **9,** 408–544 (Masson et Cie., Paris, France, 1949)
44. Grassé, P. P., and Noiret, C., *Compt. rend.*, **224,** 219 (1947)
44a. Grassi, B., and Sandias, A., *Quart. J. Microscop. Sci.*, **39,** 245–322 (1896); **40,** 1–25 (1897)
45. de Groot, A. P., and Voogd, S., *Experientia*, **10,** 384–85 (1954)
46. Haas, A., *Naturwissenschaften*, **39,** 484 (1952)
47. Haller, H. L., Acree, F., Jr., and Potts, S. F., *J. Am. Chem. Soc.*, **66,** 1659–62 (1944)
48. Haydak, M. H., *J. Econ. Entomol.*, **36,** 778–92 (1943)
49. Haydak, M. H., and Vivino, A. E., *Ann. Entomol. Soc. Am.*, **43,** 361–67 (1950)
50. Hecker, E., *Proc. Intern. Congr. Entomol. 10th Meeting* (Montreal, Que., Canada, 1956) (In press)
51. Inhoffen, H. H., *Arch. Pharm.*, **284,** 337–41 (1952)
52. Johansson, T. S. K., *Bee World*, **36,** 3–13, 21–32 (1955)
53. Kaiser, P., *Naturwissenschaften*, **42,** 303–4 (1955)
54. Kaiser, P., *Mitt. Hamburgischen Zool. Museum u. Inst.*, **54,** 129–278 (1956)
55. Kalmus, H., und Ribbands, L. R., *Proc. Roy. Soc. (London)*, [B]**140,** 50–59 (1952)
56. Karlson, P., *Vitamins and Hormones*, **14,** 227–66 (1956)
57. Kullenberg, B., *Zool. Bidr. från Uppsala*, **31,** 253–354 (1956)
57a. Lange, R., *Naturwissenschaften*, **45,** 196 (1958)
58. Lecomte, J., *Z. vergleich. Physiol.*, **32,** 499–506 (1950)
59. Lecomte, J., *Compt. rend.*, **245,** 2385–87 (1957)
60. Light, S. F., *Univ. Calif. (Berkeley) Publ. Zoöl.*, **43,** 413–54 (1944)
61. Lipke, H., and Fraenkel, G., *Ann. Rev. Entomol.*, **1,** 17–44 (1956)
62. Lüscher, M., *Z. vergleich. Physiol.*, **34,** 123–41 (1952)
63. Lüscher, M., *Naturwissenschaften*, **42,** 186 (1955)
64. Lüscher, M., *Ins. Soc.*, **3,** 119–28 (1956)
65. Lüscher, M., *Rev. suisse zool.*, **63,** 261–67 (1956)
66. Lüscher, M., *Naturwissenschaften*, **45,** 69–70 (1958)
66a. Lüscher, M., *Z. angew. Entomol.* (In press)
67. Lüscher, M., and Karlson, P., *J. Insect Physiol.*, **1,** 341–45 (1958)
68. Lukoschus, F., *Ins. Soc.*, **2,** 221–36 (1955)
69. Lukoschus, F., *Z. Bienenforsch.*, **3,** 190–99 (1956)
70. Magnus, D., *Z. Tierpsychol.*, **7,** 435–49 (1950)
71. Makino, K., Satoh, K., and Inagami, K., *Biochim. et Biophys. Acta*, **19,** 394–95 (1956)
72. Melampy, R. M., and Jones, D. B., *Proc. Soc. Exptl. Biol. N.Y.*, **41,** 382–88 (1939)

73. Müssbichler, A., *Z. vergleich. Physiol.*, **34,** 207–21 (1952)
74. Pain, J., *Ins. Soc.*, **1,** 59 (1953)
75. Pain, J., *Ins. Soc.*, **3,** 199–202 (1956)
76. Renner, M., *Naturwissenschaften,* **42,** 589 (1955)
77. Renner, M., *Z. vergleich. Physiol.* (In preparation)
78. Rhein, W. v., *Wilhelm Roux' Arch. Entwicklungsmech. Organ.*, **129,** 601–65 (1933)
79. Rhein, W. v., *Verhandl. deut. zool. Ges.* (*Wilhelmshaven*), **16,** 99–101 (1951)
80. Rhein, W. v., *Ins. Soc.*, **3,** 203–12 (1956)
81. Roth, L. M., and Willis, E. R., *Am. Midland Naturalist,* **47,** 66–129 (1952)
81a. Roth, L. M., Niegisch, A. D., and Stahl, W. H., *Science,* **123,** 670 (1956)
81b. Roth, L. M., and Stay, B., *J. Insect Physiol.*, **1,** 305–18 (1957)
82. Schneider, D., *Experientia,* **13,** 89–91 (1957)
83. Schneider, D., *Z. vergleich. Physiol.*, **40,** 8–41 (1957)
84. Schneider, D., and Hecker, E., *Z. Naturforsch.*, **11**(b), 121–214 (1956)
85. Schneider, D., and Kaissling, K.-E., *Zool. Jahrb. Abt. Anat. u. Ontog. Tiere,* **75,** 287–310 (1956)
86. Schneider, D., and Kaissling, K.-E., *Zool. Jahrb., Anat. u. Ontog. Tiere,* **76,** 223–50 (1957)
87. Schwinck, I., *Z. vergleich. Physiol.*, **35,** 167–74 (1953)
88. Schwinck, I., *Z. vergleich. Physiol.*, **37,** 19–56 (1954)
89. Schwinck, I., *Z. vergleich. Physiol.*, **37,** 439–58 (1955)
90. Schwinck, I., *Z. angew. Entomol.*, **37,** 349–57 (1955)
91. Sengün, A., *Rev. fac. sci. univ. Istanbul,* [B]**19,** 281–96 (1954)
92. Stumper, R., *Compt. rend.*, **235,** 526–28 (1952)
93. Stumper, R., *Naturwissenschaften,* **40,** 33–34 (1953)
94. Stumper, R., *Compt. rend.* **242,** 2487–89 (1956)
95. Stumper, R., *Mitt. schweiz. entomol. Ges.*, **29,** 373–80 (1956)
96. Townsend, G. F., and Lucas, C. C., *Biochem. J.*, **34,** 1155–62 (1940)
97. Valentine, I. M., *J. Exptl. Zool.*, **58,** 165–228 (1931)
98. VanBaalen, C., Forrest, H. S., and Myers, I., *Proc. Natl. Acad. Sci. U.S.*, **43,** 701–5 (1957)
99. Viscontini, M., Kühn, A., and Egelhaaf, A., *Z. Naturforsch.*, **11**(b), 501–4 (1956)
100. Viscontini, M., Loeser, E., Karrer, P., and Hadorn, E., *Helv. Chim. Acta.*, **38,** 1222–24 (1955)
101. Viscontini, M., Loeser, E., Karrer, P., and Hadorn, E., *Helv. Chim. Acta,* **38,** 2034–35 (1955)
102. Viscontini, M., and Raschig, H., *Helv. Chim. Acta,* **41,** 108–13 (1958)
103. Viscontini, M., Schmid, H., and Hadorn, E., *Experientia,* **11,** 390–92 (1955)
104. Voogd, S., *Experientia,* **11,** 181–82 (1955)
105. Voogd, S., *Experientia,* **12,** 199–201 (1956)
106. Weaver, N., *Science,* **121,** 509–10 (1955)
107. Weaver, N., *J. Econ. Entomol.*, **49,** 854–57 (1956)
108. Weaver, N., *Ann. Entomol. Soc. Am.*, **50,** 283–94 (1957)
109. Wharton, D. R. A., Miller, G. L., and Wharton, M. L., *J. Gen. Physiol.*, **37,** 461–69 (1954)

110. Wharton, D. R. A., Miller, G. L., and Wharton, M. L., *J. Gen. Physiol.*, **37**, 471–81 (1954)
111. Wharton, M. L., and Wharton, D. R. A., *J. Insect Physiol.*, **1**, 229–39 (1957)

INSECT PIGMENTS[1,2]

By R. I. T. Cromartie
University Chemical Laboratory, Cambridge, England

Introduction

The striking colours of insects have always attracted the attention of naturalists and entomologists, and an immense literature about them has accumulated; but until fairly recently very little was known of the chemical nature of the pigments responsible for them. The principal reason for this was that the classical techniques of organic chemistry were often unsuitable for the isolation of the small quantities of pigment present in the biological material. Moreover, even if pure substances could be isolated by the procedures available, a full-scale chemical study by the old methods would have involved the collection of fantastic numbers of insects. A great many of the early workers, therefore, allotted pigments to known classes of coloured compounds on the basis of colour reactions and solubility experiments performed on crude extracts. Although such results often gave valuable indications of the nature of the pigments involved, the conclusions must be accepted with reserve in the absence of more systematic investigation.

The remarkable progress in this field in the past twenty years has been principally caused by the development of new experimental methods. The advent of chromatography, which is particularly suited to coloured compounds, has enormously facilitated the purification of pigments and the separation of the complex mixtures of closely related compounds that often occur naturally. In particular, paper chromatography has made possible the separation and characterization of mixtures of pterins, for example, on a very small scale. Complementary to this has been the rapid development of visible and ultraviolet, and more recently, of infrared spectroscopy, which can now be used as routine methods for following separations and characterizing products as well as for obtaining information about the chemical constitution of individual compounds.

The aim of this review is to survey our knowledge of the chemical nature and biogenesis of the colouring matters of insects. Only their most important occurrences will be mentioned and no attempt will be made to discuss their biological significance. Emphasis will be laid on the remarkable developments since the last comprehensive review of the subject by Timon-David (87) in 1947, but some older literature will be considered in the light of recent results. Completely new groups such as the ommo-

[1] The survey of the literature pertaining to this review was completed in January, 1958.

[2] I am grateful to Professor H. Munro Fox for his criticism of the manuscript of this review.

chromes and aphins will be discussed at some length at the expense of those that have already been the subjects of reviews or monographs.

Carotenoids

The occurrence of carotenoids in a variety of insects has been claimed, but in most of the early work the only distinction drawn was between "carotene" and "xanthophyll" fractions according to behaviour in the partition test. Attention will be restricted here to cases in which individual carotenoids have been separated by chromatography and identified with some probability. Their chemistry (57) and comparative biochemistry (38) have been fully discussed in recent books. Insect carotenoids are probably always derived from the food (38), though the insect may effect minor changes in the molecule; they may occur either free or as water-soluble protein complexes.

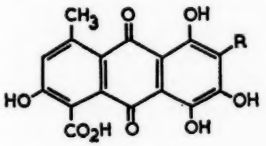
When the "carotene" fraction has been further investigated, its main or sole component has usually proved to be the orange-yellow β-carotene, the most widely distributed of all the carotenoids. Its protein complexes have been shown to be the yellow component of the "insectoverdins" (*vide infra*) of *Carausius* (*Dixippus*) *morosus* Brunner (Orthoptera) by Junge (55) and of various Lepidoptera by Hackman (44). Its distribution in locusts both as a protein complex and in the free state has been investigated by Goodwin & Srisukh (39, 40). It has been detected in *Coccinella septempunctata* Linnaeus and *Leptinotarsa decemlineata* (Say) (Coleoptera) (59) and in beeswax (88). Several occurrences among insects of the isomeric α-carotene have been reported, but apparently in no case was its identity confirmed by a mixed chromatogram. It is said to occur alone in the red spots on the female of *C. morosus* (80) and with β-carotene and lycopene in the elytra of some Coccinellidae (59); the bodies of the latter were considered to contain the rare monocyclic γ-carotene. It has also been reported to occur in *Pieris brassicae* (Linnaeus) (66), but Hackman (44) found only β-carotene and lutein in *Pieris rapae* (Linnaeus). The red fat-soluble pigment of *Pyrrhocoris apterus* (Linnaeus) that had been observed by several workers was identified by Lederer (59) as lycopene, which he also found with carotene in *C. septempunctata;* but more recently Manunta (68) could not detect it in either insect.

Several carotenoids containing oxygen have also been detected in insects. The main component of the hypophasic "xanthophyll" in the partition test is usually 3,3′-dihydroxy-α-carotene, which is of very widespread occurrence in leaves; it is sometimes known as lutein and sometimes as xanthophyll, though the latter name can be confused with the generic name for hydroxycarotenoids. Lutein has been isolated in a crystalline state by Oku (78) from the cocoon of *Bombyx mori* (Linnaeus), of which it is the principal pigment in many strains, and also from the mulberry leaves on which it feeds. Tischer (88) has shown that both propolis and ordinary beeswax contain lutein in the form of an ester with an unidentified acid.

Lutein has been shown by Junge (55) to be present as a protein complex in the yellow component of the "insectoverdins" of the integument of *Tettigonia* spp. and *Meconema varia* (Fabricius) (Orthoptera) and *Sphinx ligustri* Linnaeus (Lepidoptera); Hackman (44) has shown that both lutein and β-carotene occur as protein complexes in the blood of various Lepidoptera. The detection of two epoxycarotenoids, violaxanthin and taraxanthin, among Lepidoptera has been claimed by Manunta: taraxanthin in *P. brassicae* (66) and *B. mori,* (65) and violaxanthin in *Philosamia ricini* (Boisduval) (67). The colour test with acids used to distinguish them, is, however, of doubtful specificity, according to Eugster & Karrer (31), who have recently reinvestigated taraxanthin. Astaxanthin, 3,3′-dihydroxy-4,4′-diketo-β-carotene, is widely distributed among Crustacea, and has been shown to occur in a number of insects. The stable ketol form is red, but astaxanthin forms blue salts derived from the enediol tautomer —CHOH·CO— (red ⇆ —C(OH) = C(OH)— (blue). According to Kuhn & Sörensen (58), the bluish-green chromoprotein ovoverdin from lobsters contains as its prosthetic group astaxanthin in this form. A similar protein complex of astaxanthin was shown by Okay (75) to be responsible for the blue colour of the wings of the locust *Oedipoda caerulescens* (Linnaeus) and by Goodwin & Srisukh (39, 40) for that of some atypical blue specimens of *Locusta migratoria* (Linnaeus). Occurring with β-carotene, it caused the greenish colour of the wings of normal locusts of this and other species. The red chromoproteins of *Oedipoda miniata* (Pallas) were also found by Okay (75) to have astaxanthin as their prosthetic group, presumably in the ketol form. It has also been found in the free state by Goodwin & Srisukh in the blood and integument of locusts (39, 40).

Anthraquinones

The Coccidae produce three important dyestuffs, the colour of which is attributable to three closely related anthraquinones: that of kermes from *Kermesococcus ilicis* (Linnaeus) to kermesic acid; that of cochineal from *Dactylopius coccus* Costa to carminic acid; and that of lac dye from *Laccifer lacca* Kern to laccaic acid. Little work has been done on them since that of Dimroth and his collaborators, who proposed Formula I(R =



I

Kermesic Acid (R = —CO·CH_3)
Carminic Acid (R = —$C_6H_{11}O_5$)

—$CO \cdot CH_3$) for kermesic acid (26) and Formula I(R = —$C_6H_{11}O_5$) for carminic acid, which has the same chromophore (27); this orientation of the substituents has recently been supported on biogenetic grounds (6). The structure of the sugarlike side chain in carminic acid is unknown, but it is not glycosidically bound. The pigments of other *Dactylopius* species studied by Fester and Lexow (32) have similar spectra and are evidently closely related to carminic acid. It has recently been claimed that laccaic acid, which has been much less investigated, is in fact a mixture (94). A full account of the chemistry of these substances will be found in a recent book by Thomson (see 86). Lanigerin and strobinin are aphins (*vide infra*).

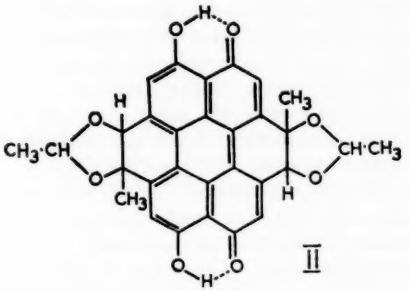
Aphins

The blood of many species of aphids contains a purplish-red pigment, which often colours the insects almost black. If the insects are crushed, the pigment undergoes a remarkable series of colour changes first described by Sorby in 1871 (84). In 1936 Blount (7) isolated an orange pigment, lanigerin, from *Eriosoma lanigerum* (Hausmann), and a red pigment, strobinin, from *Adelges strobi* Börner and suggested that they were polyhydroxyanthraquinones. Pigments that were evidently of the same type were observed in various Aphididae by Schultz (83) and Ackerman (1), who did not, however, isolate them.

The systematic chemical investigation of this unique series of natural colouring matters was begun by Todd and his collaborators (30, 51) in 1947. They showed that the blood of the living insects contains a water-soluble pigment, protoaphin; in solutions of pH greater than 5.5 it has a deep magenta colour, which changes reversibly to brownish-yellow in more acid solutions. If the aphids are crushed in an approximately neutral buffer solution, an enzyme present in them converts them to a yellow, intensely fluorescent substance, which can be extracted with ether from the aqueous solution. Treatment with acids or bases changes the colour irreversibly, first to orange and then, more slowly, to red. Corresponding to these three colours, three distinct substances can be isolated by fractional crystallization: the yellow xanthoaphin, the orange chrysoaphin, and the carmine-red erythroaphin. Unlike protoaphin, they all show an intense fluorescence, especially in ultraviolet light, and they all have characteristic absorption spectra with bands sharp enough to be seen with a hand spectroscope, by which they can be detected in the presence of one another. Xanthoaphin is very unstable, passing readily on treatment with traces of acids or bases into chrysoaphin. Chrysoaphin is more stable, but is readily converted to erythroaphin by the action of acids or bases. All the fluorescent aphins give Blount's reaction (7), which is a sensitive test for them: with acetic anhydride and a drop of sulphuric acid they give a red colour, which rapidly changes to green and then blue with a red fluorescence. Aphins have been

detected (29), in about 20 species of Aphididae and also in the genus *Adelges* (Phylloxeridae) in quantities that may reach 2 per cent of the live weight in the case of ***Tuberolachnus salignus*** (Gmelin). As protoaphin and the enzyme that converts it into xanthoaphin always occur together, the sensitivity of the tests for aphins could be increased by adding protoaphin to the pulp of the species under examination. The pigments derived from different species were designated by suffices, e.g., protoaphin-fb from *Aphis fabae* Scopoli. On this general nomenclature Blount's strobinin is erythroaphin-st and his lanigerin is chrysoaphin-ln. The corresponding aphins of the different species were very similar in most of their properties, but two series could be distinguished by their infrared spectra (53) and solubilities: the aphins-fb, with which most of the others were identical, and the aphins-sl from *T. salignus*.

In view of the instability of their precursors, most of the chemical work has been done on the stable end products, the erythroaphins, which have the molecular formula $C_{30}H_{22}O_8$. The two erythroaphins have identical visible and ultraviolet spectra and therefore the same chromophore, which was identified by degradation and synthetic experiments as that of 4,9-dihydroxyperylene-3,10-quinone (9). The arrangement of the remaining, saturated, part of the molecule is still being investigated, but the Formula II



II

Erythroaphin

is the most likely in the light of recent results (23). Erythroaphin-fb and erythroaphin-sl differ from one another only in the arrangement of this part of the molecule in space: in the former both the dioxolan rings are *cis*-fused on to the rest of the molecule; in the latter one of them has the less stable *trans*-fusion. Irradiation of the tetraacetyldihydro derivative of either yields a mixture of the two isomers together with a third (*trans-trans*) isomer corresponding to another erythroaphin that has not been found in nature (10). Conversion of erythroaphin-sl into the more stable fb-isomer can also be achieved by methods involving treatment with basic reagents (54). The erythroaphins form green salts with alkalies, which,

however, attack them further in the presence of oxygen. They are remarkably stable to acids, dissolving unchanged in cold concentrated sulphuric acid. This acid and chloroform are the only solvents in which erythroaphin dissolves freely.

The unstable intermediate pigments chrysoaphin $C_{30}H_{24}O_9$ and xanthoaphin $C_{30}H_{26}O_{10}$ differ from erythroaphin stoichiometrically by 1 H_2O and 2 H_2O respectively. Though, like erythroaphin, chrysoaphin shows the redox behaviour characteristic of quinonoid compounds, it has quite different chromophores. The ready transformations xanthoaphin → chrysoaphin → erythroaphin represent a progressive aromatization of the molecule with elimination of water. Whereas xanthoaphin-fb and chrysoaphin-fb are homogeneous, xanthoaphin-sl can be resolved by paper chromatography into two and chrysoaphin-sl into three stereoisomers; but they all give the unique erythroaphin-sl (54). Protoaphin-fb can be isolated as yellow crystals, which appear brownish-green in bulk, from aphids which have been killed in water at 70° C. to destroy the enzyme that converts it into xanthoaphin (51). Protoaphin-fb is sparingly soluble in water and in most organic solvents, but soluble in aqueous alcohol or acetone and in *N*-butanol, which extracts it from aqueous solutions. It is a surprisingly strong acid (pK 5.95) and dissolves readily in sodium bicarbonate solutions as its deep magenta salt. These properties can be utilized to purify it by counter-current distribution (23). The protoaphins owe their solubility properties to their glucosidic nature, which has been demonstrated by their hydrolysis by acids to give one molecule of glucose per molecule of protoaphin, together with small amounts of erythroaphin and large quantities of ill-defined decomposition products. In contrast to this, the enzyme present in the insects converts protoaphin into xanthoaphin almost quantitatively; but it is not a simple hydrolysis since one atom of oxygen per molecule is taken up from the air in the process (23). Elementary analysis of protoaphin-fb suggests a formula $C_{36}H_{40}O_{16}$, differing from xanthoaphin by $C_6H_{12}O_6$ (glucose) + 2H; but its constitution has not yet been elucidated.

A related but distinct series of pigments occurring in *Hamamelistes spinosus* Shimer and *Hamamelistes betulae* (Mordvilko), has been investigated by Macdonald (63). The blood of these aphids contains a water-soluble pigment heteroaphin; this is converted by enzymes present in the insects into another red pigment rhodoaphin, which can be extracted by ether. The rhodoaphins are closely related to the erythroaphins: they give Blount's colour reaction, and they can be reduced to an eryythroaphin with zinc dust and acetic acid. Their elementary analyses suggest a formula $C_{30}H_{22}O_{11}$, isomeric with trihydroxyerythroaphins, but no further chemical work has been done on them. The heteroaphins can be converted to rhodoaphins by mild acid hydrolysis as well as by action of the enzymes present in the insect, for which the protoaphins are not substrates. The red solutions of the heteroaphins undergo a reversible colour change to green with alkalies.

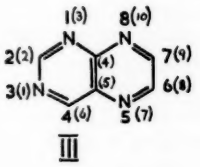
Anthoxanthins and Anthocyanins

Only one insect pigment belonging to either of these classes has actually been isolated: Thomson (85) obtained a crystalline anthoxanthin from the wings of *Melanargia galathea* (Linnaeus) (Lepidoptera) and showed that it was identical with that of its food plant, *Dactylis glomerata* Linnaeus. A large number of Lepidoptera have been tested for the presence of anthoxanthins by the yellow coloration with ammonia by Ford (34), who used the results taxonomically. Flavonoid compounds were said by Manunta (64) to occur in some races of *Bombyx mori* (Linnaeus), but the evidence for them is very inconclusive.

There is little real evidence for the occurrence of anthocyanins in insects. The best supported case seems to be that of *Cionus olens* Fabricius, which was shown by Hollande (50) to contain a pigment with similar properties to one found in its food plant *Verbascum nigrum* Linnaeus. The acridoxanthins described by Chauvin (21) in many Orthoptera are ommochromes. The pigments of *Tritogenaphis rudbeckiae* (Fitch) and *Pterocomma smithiae* (Monell) (37) (both Aphididae) are probably aphins. Halochromic pigments have been observed in Hemiptera of other families: the red pyrrhocorine of *Pyrrhocoris apterus* (Linnaeus) and related Lygaeidae by Manunta (68); the blue pigment of *Nezara viridula* (Linnaeus) and other Pentatomidae by Okay (76) and Hackman (44); and yellow pigments in species of several families by Palmer & Knight (79). All have been tentatively described as anthocyanins or flavones, but have not been further investigated.

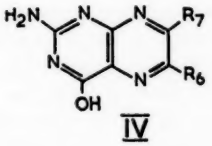
Pterins

The pterins, which form the most numerous group of insect pigments and also occur in many other classes of animals, are derived from the heterocyclic system pteridine (Formula III[3]). The extensive literature on



III

Pteridine



IV

Pterins

them was reviewed by Purrmann (81); more recently it has been surveyed by Albert (3) from a chemical and by Ziegler-Günder (105) from a

[3] The numbering shown is commonly used by English-speaking authors; that in parentheses is customary on the Continent.

biological point of view. Emphasis will therefore be laid on the most recent contributions to this rapidly developing subject.

Of the sparingly soluble pterins first observed in the wings of Pieridae, the constitutions of leucopterin (Formula IV: $R_6 = OH$; $R_7 = OH$), xanthopterin (Formula IV: $R_6 = OH$; $R_7 = H$), isoxanthopterin (Formula IV: $R_6 = H$; $R_7 = OH$), and chrysopterin (Formula IV: $R_6 = OH$; $R_7 = CH_3$) have been firmly established by degradation and synthesis (3, 81). The orange-red pigment of the Pieridae, erythropterin, which was considered by Purrmann & Eulitz (82) to be Formula IV [$R_6 = OH$; $R_7 = -C(OH) = C(OH) - CH_2OH$], has been synthesized (91). Nevertheless, this formula does not completely explain the spectrum and properties of the pigment; and Tschesche & Barkmeier (90) have recently suggested a tricyclic formula that might exist in equilibrium with it. The occurrence of this group of pterins was surveyed by Becker (4).

With the development of paper chromatography and of ultraviolet spectrophotometry, it has become possible to separate and characterize the pterins that are present in small quantities in individual organs of insects, and in many cases to identify them by comparison with synthetic substances. The pioneer work was done by Hadorn & Mitchell (47) on *Drosophila melanogaster* Meigen and by Hadorn & Kühn (46) on *Anagasta kühniella* (Zeller) (=*Ephestia kühniella* Zeller), which were chosen so that insight into the metabolism of the pterins might be gained by comparing the numerous mutants of these insects. More recently other Lepidoptera have been studied by Müller (72) and by Grossbach (43). By developing paper chromatograms with two different solvent systems in two directions at right angles, a characteristic two-dimensional pattern of fluorescent spots was obtained for each type of material. In the wild type of *Drosophila* Hadorn & Mitchell (47) distinguished seven spots (Fl-1 to Fl-7), several of which have been isolated or identified.

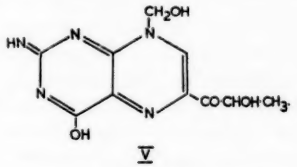
Fl-1 is the red pigment that occurs together with brown ommochromes in the eyes of the wild type of *Drosophila* and without them in mutants *v* and *cn*. It had already been isolated in a crude form by Lederer (60), who suggested that it was a pterin and called it drosopterin, and by Chan, Heymann & Clancy (20), who showed that it was a mixture. Recently Viscontini, Hadorn & Karrer (96) have separated and purified three components of the mixture and called them drosopterin, isodrosopterin, and neodrosopterin. They all have very similar spectra; all are unstable to acids and alkalies; and all are degraded by oxidation to Formula IV($R_6 = COOH$; $R_7 = H$), which shows that they are in fact pterins and have a side chain on C-6.

The violet-fluorescent Fl-3 has been identified (36, 99) as isoxanthopterin, which also occurs in *Anagasta* (=*Ephestia*) (97) (spot *g*) and other Lepidoptera (43). It has been isolated from *B. mori* by Nawa and his collaborators (73) and by Tschesche & Korte (92), who showed that it was

one component of "fluorescyanine," an ill-defined mixture obtained by previous workers from *B. mori* and other sources. The fluorescyanine from *B. mori* probably also contains a derivative of isoxanthopterin with a side chain on C-6; but apparently it is not ichthyopterin (Formula IV: $R_6 = -CH_2 \cdot CO_2H$; $R_7 = OH$), which occurs in certain fish and with which "fluorescyanine" had been erroneously identified. Experiments on mutants by Hadorn (45) show that isoxanthopterin plays an important part in the metabolism of pterins in *Drosophila;* and Hadorn & Schwink (48) have found a mutant ry^2 lacking isoxanthopterin, which is nonautonomous for the red eye pigments. It is possible that it is a biosynthetic precursor of them. Xanthopterin appears in the fluorescent pattern of *Anagasta* (97) (spot *h*) and other Lepidoptera (43).

The blue-fluorescent Fl-4 has been resolved into two, Fl-4A and Fl-4B, by Forrest & Mitchell (36). Fl-4A was shown by degradation and synthesis to be Formula IV ($R_6 = -CHOH \cdot CHOH \cdot CH_3$; $R_7 = H3$; it is the same as the HB_2 of Viscontini, Loeser, Karrer & Hadorn (98), who proposed and later withdrew a different formula for it. Fl-4B (HB_1 of the Swiss school) was identified as 2-amino-4-hydroxypteridine (Formula IV: $R_6 = R_7 = H$). Its 6-carboxy derivative (Formula IV: $R_6 = -COOH$; $R_7 = H$) was also detected by Viscontini *et al.* (98) as a component of Fl-4. The spots k_2 and k_1 in *Ephestia* correspond, according to Viscontini, Kühn & Egelhaaf (97), to Fl-4A and Fl-4B respectively.

Fl-5 is a yellow pigment with a yellow fluorescence, which occurs with the drosopterins in the eyes of wild type *Drosophila* and also in the testes. It is fairly certain that it is a biogenetic precursor of the drosopterins, since it accumulates in much larger quantities in the eyes of the mutant sepia (*se*) from which drosopterins are absent. Forrest & Mitchell (35) were able to isolate it from the flies in a pure crystalline state in sufficient quantity for degradative experiments. Like riboflavin, which it resembles in many of its properties, it was very sensitive to light, being degraded to Formula IV ($R_6 = CO_2H$; $R_7 = H$), which was also formed on oxidation. The formation of lactic acid on photolysis and of acetaldehyde on periodate



V

"Sepia" Pigment

oxidation suggested that it contained a lactyl side chain, which Forrest and Mitchell placed at N-8 with a free carboxyl group at C-6 in their proposed formula. As Ziegler-Günder (104), who isolated the same compound from

frogs, showed by paper electrophoresis that it contains no free carboxyl group, the more recent Formula V, proposed on other chemical and spectroscopic grounds by Fidler & Wood (33), seems much more likely. This would also fit in well with the suggestion of De Vincentiis (95) that Fl-4A, which also accumulates to a smaller extent in *se* flies, is its biogenetic precursor. The "xanthopterin-B" from the mutant *lem* of *B. mori* has been shown by Nawa & Taira (74) to consist mainly of Fl-5. Aruga & Kawase (2) have shown that the $+^{lem}$ silkworms contain an enzyme that destroys one component of their "xanthopterin-B." It seems possible that some of the yellow pigments of insects referred to in the literature as riboflavin may in fact be this substance, which is very similar in its spectrum, fluorescence, sensitivity to light, and chromotgraphic behaviour. The problem of distinguishing between them has been discussed by Ziegler-Günder (104) who showed that the "sepia" pigment has no vitamin B_2 activity and by Daneel & Eschirch (24), who investigated individual organs of *Drosophila* and other Diptera. They showed that the pigment of the Malpighian tubes was in fact riboflavin, in accord with the earlier work of Busnel & Drilhon (11) on *Attacus* spp. (Lepidoptera) and of Metcalf (70) on *Periplaneta americana* (Linnaeus), who demonstrated remarkable accumulations of riboflavin in these organs by biological assays that are probably reasonably specific. According to De Vincentiis (95), riboflavin mononucleotide is responsible for the ubiquitous Fl-2.

"Insectoverdins" and Bile Pigments

The key to the nature of the green pigments that are common among insects, which many early investigators had thought to be chlorophyll, was discovered by Przibram & Lederer (80) in 1933. They showed that in several locusts the green colour was due to a mixture of a yellow and a blue chromoprotein: the prosthetic group of the yellow one was in each case a carotenoid; that of the blue one was a bile pigment, which they isolated in a crystalline state from *Carausius morosus* Brunner but did not characterize or investigate further. The name "insectoverdin" was proposed for such mixtures in 1941 by Junge (55), who extracted it from the integument of several species of Orthoptera, including *C. morosus*, and of *Sphinx ligustri* Linnaeus (Lepidoptera): in each case the prosthetic group of the yellow chromoprotein was a carotenoid and that of the blue a bile pigment, which he suggested on spectroscopic grounds to be a mesobiliverdin (synonymous with glaucobilin). Very similar bile pigments were found in the "insectoverdins" of solitary locusts by Goodwin & Srisukh (42) and of the haemolymph of various Lepidoptera by Hackman (44). The blue chromoproteins of the wings of Pieridae were investigated by Wieland and his collaborators (100), who isolated the prosthetic group, pterobilin, from *Pieris brassicae* (Linnaeus) in a crystalline state (101). They tentatively proposed a formula of the biliverdin type for it, but the evidence has been

reconsidered by Tixier (89) and by Lemberg & Legge (61), who conclude that it is a mesobiliverdin. Although the spectroscopic data on all these bile pigments are collected in Hackman's paper (44), the questions of their possible identity with one another and the details of their molecular structure remain open.

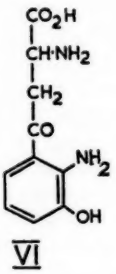
A number of other bile pigments and porphyrin derivatives have been found in insects. Wigglesworth (102) has shown that *Rhodnius prolixus* Stål and other bloodsucking insects convert haemoglobin to biliverdin and a number of other degradation products. The work of Metcalf (71) on *Anasa tristis* (DeGeer) suggests that a similar catabolism of chlorophyll may be possible. Very recently, the respiratory pigment cytochrome-C, which has long been known to occur in insects, has been isolated from *B. mori* by Tuppy (93); its peptide chain has one amino acid different from that of mammalian cytochrome-C. The red and brown pigments of *Vanessa spp.* investigated by von Linden (62) are not bile pigments but ommochromes (*vide infra*).

Ommochromes

The ommochromes are a group of closely related brown, yellow, or red pigments, which are widely distributed among arthropods, especially in the eyes, but also in other parts of the body. Their distribution was discussed in detail by Becker (5), who first recognized them as a homogeneous class distinct from previously known types of natural colouring matters. It included the ommins and the ommatins, two sorts of pigment that he had investigated in the eyes of insects. The group properties by which he characterized and distinguished between them will be discused later, in connection with chemistry of the individual ommochromes. In nature, they often occur as chromoproteins from which they can be liberated by denaturation of the protein. The elucidation of the chemical nature of these intractable substances has been enormously helped by the classic genetic studies on mutations affecting the eye colour of *Drosophila melanogaster* Meigen and *Anagasta kühniella* (Zeller), which have been reviewed by Karlson (56). The results showed clearly that the ommochromes are formed in the insect from tryptophan, which is converted by a chain of genetically controlled processes to kynurenine and then to 3-hydroxykynurenine (Formula VI), the immediate precursor or chromogen of the ommochromes. They are best defined in terms of this metabolic origin, as was originally proposed by Becker, especially since tracer studies now provide an experimental criterion for it. The ommochromes of Orthoptera have been studied by Okay (77) and by Goodwin & Srisukh (41), who confirmed that Chauvin's acridoxanthins (21) were ommochromes.

In the light of these results, Butenandt and his collaborators began in 1950 a thorough chemical study of the ommochromes. The best source for their extraction on an adequate scale was found to be the red moulting

fluid of *Vanessa urticae* (Linnaeus) (Lepidoptera), which had already been investigated by von Linden (62) and Becker (5). Paper chromatography revealed that Becker's preparations were all mixtures of several substances, but by fractional precipitation and large-scale circular paper



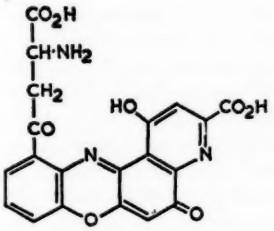
VI

3-Hydroxykynurenine

chromatography, two pure ommochromes, xanthommatin and rhodommatin, were isolated and characterized by their R_F values and infrared spectra (17). A third compound, ommatin-C, isolated at the same time, later proved to be an artefact derived from the decomposition of rhodommatin. Very recently a third natural ommatin, ommatin-D, has been obtained from the same source (14). These three compounds were all found to be ommochromes on the definition given above by injection of C^{14}-labelled tryptophan and kynurenine into *Vanessa* larvae: the three ommatins separated by paper chromatography from the moulting fluid were all radioactive (13). A direct connection with the eye pigments and therefore with the genetic results was established by isolating crystalline xanthommatin from the eyes of *Calliphora erythrocephala* (Meigen) (16). Xanthommatin shows the remarkable redox behaviour regarded by Becker as characteristic of the ommatins: the normal yellowish-brown form is reduced by sulphur dioxide or sodium dithionite, not to a colourless leuco compound, but to the deep-red hydroxanthommatin, which was obtained in a crystalline state; it undergoes oxidation in alkaline or strongly acid solution back to the brown form, though it is unchanged at intermediate pH (17). Both states of oxidation occur in nature, sometimes in the same species in different parts or at different stages of development (12). Rhodommatin and ommatin-D, on the other hand, occur naturally in a red reduced form corresponding to hydroxanthommatin: they are oxidized only very slowly by air or more rapidly by nitrite or ferricyanide to a brown form, which is readily reduced back to the original red form. The ultraviolet and visible spectra of the three ommatins are almost identical in corresponding states of oxidation, showing that they are closely related chemically (14). The ommatins are insoluble

in water and in the common organic solvents in both states of oxidation (17). The oxidized forms are somewhat soluble in dilute mineral acids, and all are freely soluble in concentrated sulphuric acid to give violet solutions and in methanolic hydrochloric acid, from which hydroxanthommatin can be recrystallized. Being carboxylic acids, the ommatins are soluble in buffer solutions of pH 5.5; but at pH 8 the oxidized forms decompose fairly rapidly, and the reduced forms more slowly, to colourless products (18): this lability to alkali was another of Becker's characteristic properties of the ommatins. Two substances can be detected among the products of alkaline degradation of all three ommatins by their fluorescence on paper chromatograms: xanthurenic acid and 2-amino-3-hydroxyacetophenone, which are also formed from 3-hydroxykynurenine under the same conditions. The same products were also formed by the alkaline degradation of an ommin and can therefore be regarded as characteristic of the ommochromes as a group (14). A further indication of a close chemical relation between xanthommatin and its chromogen, 3-hydroxykynurenine, was furnished by the observation that it is a substrate for the enzyme kynureninase, which is specific for the kynurenine side chain (18).

It was found that the conversion of 3-hydroxykyneurenine into xanthommatin could be carried out by a simple reaction *in vitro:* oxidation with potassium ferricyanide at pH 7.1 followed by reduction with sulphur dioxide furnished crystalline hydroxanthommatin in 90 per cent yield; this synthesis made xanthommatin much more readily accessible. Considerations of the elementary analysis and ultraviolet and visible spectrum of xanthommatin and of the stoichiometry of its formation from 3-hydroxykynurenine, during which ammonia is liberated, led to the proposal of Formula VII, in which two molecules of 3-hydroxykynurenine have condensed to a phenoxa-

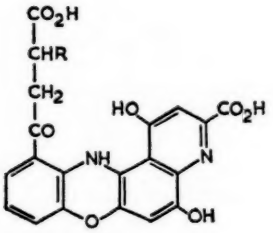


VII

Xanthommatin

zone and one of the side chains has subsequently cyclized to form a pyridine ring. This constitution was placed beyond doubt by an unambiguous synthesis from 3-hydroxykynurenine and a compound already containing this

ring (19). From their spectra, rhodommatin and ommatin-D must contain the same tetracyclic chromophore as xanthommatin, from which they probably differ in the side chain since they are not substrates for kynureninase (14). Hydroxanthommatin is formed from xanthommatin by the uptake of two atoms of hydrogen and has the constitution of Formula VII (R = NH_2) (19). As rhodommatin and ommatin-D are not substrates for kyneureninase they differ from xanthommatin in the side chain, but they have the same tetracyclic chromophoric system. Rhodommatin is probably Formula VIII (R = OH) (14).



VIII

Hydroxanthommatin (R = NH_2)
Rhodommatin (R = OH)

3-Hydroxykynurenine can also be oxidized enzymically to xanthommatin by tyrosinase in the presence of dopa or catechol, although it is not itself a substrate for tyrosinase; it is evidently oxidized by the *o*-quinones formed by the action of tyrosinase on its own substrates (15). If dopa is used the product is a mixture of xanthommatin and tyrosinemelanin, which can be separated by paper chromatography; the "red melanin" obtained by Inagami (52) from the aka-aka (*rb*) mutant of *B. mori*, which contains an abnormal amount of 3-hydroxykynurenine, is probably a similar mixture. It is quite possible that the last stage of the formation of the ommochromes in nature takes place by some such unspecific redox reaction rather than under the influence of a special enzyme. In the eyes, however, the protein granules on which the ommochromes are deposited seem from the work of Hanser on mutants of *Anagasta* to play a decisive part in their formation (49).

Because of their particularly intractable nature, less work has been done on the ommins than on the ommatins, but recently Butenandt and his collaborators (12) have isolated ommins from the heads of *B. mori* and a number of other sources. As no difference could be detected between the ommins from different sources, which appeared to be homogeneous, it is quite possible that there is only one ommin. It dissolves in methanolic hydro-

chloric acid to give a reddish-violet solution, which like rhodommatin undergoes a very slow reversible oxidation in air to a yellow oxidized form. It is also slowly degraded by alkali, as mentioned earlier. Thus, it shows the two properties regarded originally as characteristic of ommatins, redox behaviour and lability to alkalies, to the same extent as rhodommatin. Its solubility properties and absorption spectrum suggest that it has a higher molecular weight and more extensive chromophoric system than the ommatins; but there seems no doubt that the ommins are one or more definite compounds and not indefinite ploymers like melanin.

Melanins

Unlike all the other pigments discussed in this article, the melanins are polymeric in nature, so that it is impossible even in principle to isolate them as definite chemical compounds. They have been reviewed at length by Mason (69) and those of the insect cuticle have been discussed recently by Wigglesworth (103) and by Dennell (25). The best-defined melanins, those derived from dopa by the action of tyrosinase, have recently been discussed by Cromartie & Harley-Mason (22) and therefore will not be further considered here.

LITERATURE CITED

1. Ackerman, L., *J. Exptl. Zool.*, **44,** 1–61 (1926)
2. Aruga, H., and Kawase, S., *J. Sericult. Sci. Japan*, **23,** 99–107 (1954)
3. Albert, A., *Fortschr. Chem. org. Naturstoffe*, **11,** 350–403 (1954)
4. Becker, E., *Z. Morphol. Ökol. Tiere*, **32,** 672–751 (1937)
5. Becker, E., *Z. Induktive Abstammungs- u. Vererbungslehre*, **80,** 157–204 (1942)
6. Birch, A. J., and Donovan, F. W., *Australian J. Chem.*, **8,** 529–33 (1955)
7. Blount, B. K., *J. Chem. Soc.*, 1034 (1936)
8. Brown, B. R., Ekstrand, T., Johnson, A. W., Macdonald, S. F., and Todd, A. R., *J. Chem. Soc.*, 4925–28 (1952)
9. Brown, B. R., Calderbank, A., Johnson, A. W., Joshi, B. S., Quayle, J. R., and Todd, A. R., *J. Chem. Soc.* 959–65 (1955)
10. Brown, B. R., Johnson, A. W., Quayle, J. R., and Todd, A. R., *J. Chem. Soc.* 1144–53 (1955)
11. Busnel, R. G., and Drilhon, A., *Compt. rend. soc. biol.*, **137,** 752–53 (1943)
12. Butenandt, A., *Angew. Chem.*, **69,** 16–23 (1957)
13. Butenandt, A., and Beckmann, R., *Z. physiol. Chem.*, **301,** 115–17 (1955)
14. Butenandt, A., Bieckert, E., and Beckmann, R., *Ann. Chem. Liebigs*, **607,** 207–15 (1957)
15. Butenandt, A., Bieckert, E., and Linzen, B., *Z. physiol. Chem.*, **305,** 284–89 (1956)
16. Butenandt, A., and Neubert, G., *Z. physiol. Chem.* **301,** 109–14 (1955)
17. Butenandt, A., Schiedt, U., and Bieckert, E., *Ann. Chem. Liebigs*, **586,** 217–28 (1954)
18. Butenandt, A., Schiedt, U., and Bieckert, E., *Ann. Chem. Liebigs*, **586,** 229–39 (1954)
19. Butenandt, A., Schiedt, U., Bieckert, E., and Cromartie, R. I. T., *Ann. Chem. Liebigs*, **590,** 75–90 (1954)
20. Chan, F. L., Heymann, H., and Clancy, C. W., *J. Am. Chem. Soc.* **73,** 5448–49 (1951)
21. Chauvin, R., *Compt. rend. soc. biol.*, **135,** 334–35 (1941)
22. Cromartie, R. I. T., and Harley-Mason, J., *Biochem. J.*, **66,** 713–20 (1957)
23. Cromartie, R. I. T., and Todd, A. R. (Unpublished results)
24. Daneel, R., and Eschirch, B., *Z. Naturforsch.*, **11**(b), 105–10 (1956)
25. Dennell, R., *Biol. Revs. Cambridge Phil. Soc.*, **33,** 178–96 (1958)
26. Dimroth, O., and Fick, R., *Ann. Chem. Liebigs*, **411,** 315–38 (1916)
27. Dimroth, O., and Kämmerer, H., *Ber. deut. chem. Ges.*, **53,** 471–80 (1920)
29. Duewell, H., Human, J. P. E., Johnson, A. W., Macdonald, S. F., and Todd, A. R., *J. Chem. Soc.*, 3304–9 (1950)
30. Duewell, H., Johnson, A. W., Macdonald, S. F., and Todd, A. R., *J. Chem. Soc.*, 485–90 (1950)
31. Eugster, C. H., and Karrer, P., *Helv. Chim. Acta*, **40,** 69–79 (1957)
32. Fester, G. A., and Lexow, S., *Anales soc. cient. arg.*, **135,** 89–96 (1943)
33. Fidler, W. E., and Wood, H. C. S., *J. Chem. Soc.* 4157–62 (1957)
34. Ford, E. B., *Proc. Roy. Entomol. Soc. (London)*, [A]**16,** 65–90 (1941)
35. Forrest, H. S., and Mitchell, H. K., *J. Am. Chem. Soc.*, **76,** 5656–62 (1954)
36. Forrest, H. S., and Mitchell, H. K., *J. Am. Chem. Soc.*, **77,** 4865–69 (1955)
37. Glaser, R. W., *Psyche*, **24,** 30 (1917)

38. Goodwin, T. W., *The Comparative Biochemistry of the Carotenoids* (Chapman and Hall, London, England, 356 pp., 1952)
39. Goodwin, T. W., *Biochem. J.*, **45,** 472–79 (1949)
40. Goodwin, T. W., and Srisukh, S., *Biochem. J.*, **45,** 263–68 (1949)
41. Goodwin, T. W., and Srisukh, S., *Biochem. J.*, **47,** 548–54 (1950)
42. Goodwin, T. W., and Srisukh, S., *Biochem. J.*, **48,** 199–203 (1951)
43. Grossbach, U., *Z. Naturforsch.* **12**(b), 462 (1957)
44. Hackman, R. H., *Arch. Biochem. Biophys.*, **41,** 166–74 (1952)
45. Hadorn, E., *Experientia,* **10,** 483–84 (1954)
46. Hadorn, E., and Kühn, A., *Z. Naturforsch.*, **8**(b), 582–89 (1953)
47. Hadorn, E., and Mitchell, H. K., *Proc. Natl. Acad. Sci. U.S.*, **37,** 650–65 (1951)
48. Hadorn, E., and Schwinck, I., *Nature,* **177,** 940–41 (1956); *Z. Induktive Abstammungs- u. Vererbungslehre,* **87,** 528–53 (1956)
49. Hanser, G., *Z. Naturforsch.*, **1,** 396–99 (1946)
50. Hollande, A. C., *Arch. zool. exptl. gen.*, **51,** 53–58 (1913)
51. Human, J. P. E., Johnson, A. W., Macdonald, S. F., and Todd, A. R., *J. Chem. Soc.*, 477–85 (1950)
52. Inagami, K., *Nature,* **174,** 1105 (1954); *J. Sericult. Sci. Japan,* **23,** 228–32 (1954), **24,** 20–26, 80–85 (1955)
53. Johnson, A. W., Quayle, J. R., Robinson, T. S., Sheppard, N., and Todd, A. R., *J. Chem. Soc.*, 2633–38 (1951)
54. Johnson, A. W., Todd, A. R., and Watkins, J. C., *J. Chem. Soc.*, 4091 (1956)
55. Junge, H., *Z. physiol. Chem.*, **268,** 179–86 (1941)
56. Karlson, P., *Ergeb. Enzymforsch.*, **13,** 85–206 (1954)
57. Karrer, P., and Jucker, E., *The Carotenoids* (Elsevier, Amsterdam, Netherlands, 384 pp., 1950)
58. Kuhn, R., and Sörensen, N. A., *Ber. deut. chem. Ges.*, **71,** 1879–88 (1938)
59. Lederer, E., *Bull. soc. chim. biol.*, **20,** 567–610 (1938)
60. Lederer, E., *Biol. Revs. Cambridge Phil. Soc.*, **15,** 273–306 (1940)
61. Lemberg, R., and Legge, J. W., *Haematin Compounds and Bile Pigments* (Interscience Publishers, Inc., New York, N.Y., 749 pp., 1949)
62. Linden, M. von, *Arch. ges. Physiol. Pflüger's,* **98,** 1–89 (1903); *Ann. sci. nat. Zool.*, **20,** 295–363 (1904)
63. Macdonald, S. F., *J. Chem. Soc.*, 2378–81 (1954)
64. Manunta, C., *Mem. reale accad. nazl. Lincei, Classe sci. fis. mat. e nat.*, [6]**6,** 75–160 (1935)
65. Manunta, C., *Boll. soc. ital. biol. sper.*, **12,** 626–28 (1937)
66. Manunta, C., *Atti reale accad. Italia. Rend. classe sci. fis. mat. e nat.*, [7]**3,** 151 (1941)
67. Manunta, C., *Sci. Genet. (Turin)*, **2,** 273–79 (1942)
68. Manunta, C., *Boll. soc. ital. biol.* **26,** 295–98 (1950)
69. Mason, H. S., *Advances in Enzymol.*, **16,** 105–84 (1955)
70. Metcalf, R. L., *Arch. Biochem.*, **2,** 55–62 (1943)
71. Metcalf, R. L., *Ann. Entomol. Soc. Am.*, **38,** 397–402 (1945)
72. Müller, G., *Z. Naturforsch.*, **11**(b), 221–22 (1956)
73. Nawa, S., Goto, M., Matsuura, S., Kakizawa, H., and Hirata, Y., *J. Biochem. (Tokyo)*, **41,** 657–60 (1954)
74. Nawa, S., and Taira, T., *Proc. Japan Acad.*, **30,** 632–35 (1954)

75. Okay, S., *Rev. fac. sci. univ. Istanbul,* [B]**12,** 1–8 (1947); *Bull. soc. zool. France,* **74,** 11–15 (1949)
76. Okay, S., *Rev. fac. sci. univ. Istanbul,* [B]**12,** 89–106 (1947)
77. Okay, S., *Commun. fac. sci. univ. Ankara,* **1,** 178–86 (1948)
78. Oku, M., *Bull. Agr. Chem. Soc. Japan,* **6,** 40–49 (1930); *Chem. Abstr.* **24,** 3834 (1930)
79. Palmer, L. S., and Knight, H. H., *J. Biol. Chem.,* **59,** 451–55 (1924)
80. Przibram, H., and Lederer, E., *Anz. Akad. Wiss. Wien. Math.-natur. Klasse,* **70,** 163–65 (1933)
81. Purrmann, R., *Fortschr. Chem. org. Naturstoffe,* **4,** 64–86 (1945)
82. Purrmann, R., and Eulitz, F., *Ann. Chem. Liebigs.,* **559,** 169–74 (1948)
83. Schultz, F. N., *Biochem. Z.,* **127,** 112–19 (1922)
84. Sorby, H. C., *Quart. J. Microscop. Soc.* **11,** 352–61 (1871)
85. Thomson, D. L., *Biochem. J.,* **20,** 73–75, 1026–27 (1926)
86. Thomson, R. H., *Naturally Occurring Quinones* (Butterworth, London, England, 302 pp., 1957)
87. Timon-David, J., *Année Biol.* [3]**23,** 237–71 (1947)
88. Tischer, J., *Z. physiol. Chem.,* **267,** 14–22 (1940)
89. Tixier, R., *Bull. soc. chim. biol.,* **27,** 621–26 (1945)
90. Tschesche, R., and Barkmeier, H., *Ber. deut. chem. Ges.,* **88,** 976–83 (1955)
91. Tschesche, R., and Korte, F., *Ber. deut. chem. Ges.,* **84,** 77–83 (1951)
92. Tschesche, R., and Korte, F., *Ber. deut. chem. Ges.,* 87, 1713–19 (1954)
93. Tuppy, H., *Z. Naturforsch.,* **12**(b), 784–86 (1957)
94. Venkataraman, K., *Festschrift Arthur Stoll,* 360–75 (Birkhäuser, Basel, Switzerland, 911 pp., 1957)
95. Vincentiis, M. De, *Ricerca sci.,* **26,** 2735–38 (1956)
96. Viscontini, M., Hadorn, E., and Karrer, P., *Helv. Chim. Acta,* **40,** 579–85 (1957)
97. Viscontini, M., Kühn, A., and Egelhaaf, A., *Z. Naturforsch.,* **11**(b), 501–4 (1956)
98. Viscontini, M., Loeser, E., Karrer, P., and Hadorn, E., *Helv. Chim. Acta,* **38,** 1222–24, 2034–35 (1955)
99. Viscontini, M., Schoeller, M., Loeser, E., Karrer, P., and Hadorn, E., *Helv. Chim. Acta,* **38,** 397–401 (1955)
100. Wieland, H., and Kotschmar, A., *Ann. Chem. Liebigs,* **530,** 152–65 (1937)
101. Wieland, H., and Tarrter, A., *Ann. Chem. Liebigs,* **545,** 197–208 (1940)
102. Wigglesworth, V. B., *Proc. Roy. Soc. (London),* [B]**131,** 313–39 (1943)
103. Wigglesworth, V. B., *Ann. Rev. Entomol.,* **2,** 37–54 (1957)
104. Ziegler-Günder, J., *Z. Naturforsch.,* **11**(b), 493-500 (1956)
105. Ziegler-Günder, J., *Biol. Revs. Cambridge Phil. Soc.,* **31,** 313–48 (1956)

TAXONOMIC PROBLEMS WITH CLOSELY RELATED SPECIES[1,2]

By W. J. Brown
Insect Systematics and Biological Control Unit, Entomology Division, Ottawa, Canada

With the advent of the "new systematics," there came a greater appreciation of the need to make taxonomy as objective as possible [Huxley (46)]. The publication in 1937 of the first edition of Dobzhansky's *Genetics and the Origin of Species* organized the work on population genetics and greatly stimulated interest in the nature of species. The result has been a large number of contributions to the theory of speciation and an increasing effort by taxonomists to apply this new thought in their practical work, especially in that pertaining to closely related forms. This paper is not a review of that literature; it is instead an attempt to review the problems presented by closely related species as special problems in insect taxonomy. The following authors offer comprehensive treatments on the nature of species: Dobzhansky (26), Mayr (52, 53, 56), Stebbins (73), Emerson *et al.* (3) Jepsen *et al.* (47), and Mayr, Linsley & Usinger (57). These authors give extensive bibliographies. Mayr, Linsley & Usinger (57) also treat taxonomic procedure extensively. Important papers on special topics are cited in the discussions below; most of them include useful bibliographies.

The common names used in the section that follows are names approved by the Entomological Society of America [Sailer (71)]. Following are explanations of some of the terms used below. "Form" is used as a neutral term and may refer to any variant individual or to any specific or infraspecific unit. "Population" and "natural population" refer to the individuals of a single locality that potentially form a single interbreeding community. "Sympatric" forms are those the ranges of which overlap, and "allopatric" forms are those the ranges of which do not. A "cline" is a character gradient, the gradual and continuous change of a character through a linear series of populations. "Physiological" is used in a broad sense; all characters of forms other than the morphological are considered physiological. "Ecological" is used in a broad sense and includes all that pertains to the relationship of a population to its environment; temporal isolation and spatial isolation not maintained by physical barriers are considered types of ecological isolation.

The Problems

It is self-evident to the naturalist that plants and animals in any restricted region occur not as a chaotic mass of intergrading groups of indi-

[1] The survey of literature pertaining to this review was completed in April, 1958.

[2] Contribution No. 3817, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.

viduals but as a finite number of distinct units, each of which is an independent breeding population. These populations are species. Such populations of one region may resemble one another so closely that they are difficult to segregate, and it is frequently difficult to relate properly more or less similar populations from different regions. These are the problems commonly presented by closely related forms.

During the last quarter-century, a number of important pests of North America were distinguished from their closest allies for the first time. Examples are the cotton aphid (*Aphis gossypii* Glover), screw-worm [*Callitroga hominivorax* (Coquerel)], bronze birch borer (*Agrilus anxius* Gory), prairie grain wireworm [*Ctenicera aeripennis destructor* (Brown)], tuber flea beetle (*Epitrix tuberis* Gentner), ash-gray blister beetle [*Epicauta fabricii* (Le Conte)], eastern and western raspberry fruitworms (*Byturus rubi* Barber and *Byturus bakeri* Barber), and the jack-pine budworm (*Choristoneura pinus* Freeman). In 1944 the rice weevil [*Sitophilus oryza* (Linnaeus)] was reported to be composite in England by Richards (67) and in Australia by Birch (9). Although the rice weevil is the most important pest of stored grain in the United States, it was not shown to be composite there until 1957 [Floyd & Newsom (33)]. Gurney & Brooks (42) recently concluded that the Rocky Mountain grasshopper [*Melanoplus spretus* (Walsh)] and the migratory grasshopper [*Melanoplus mexicanus* (Saussure)], which have been considered phases of one species, are specifically distinct, that the migratory grasshopper is a complex of five species, and that one of these species is divisible into three subspecies. In all of these and in most other examples of recently revised taxonomy of closely related species, the insects themselves have not presented unusually difficult taxonomic problems. The delay in segregating the species has been due to inadequate concepts, methods, and materials of the taxonomists and, most of all, to lack of interest in the taxonomic aspects of their problems by entomologists in general.

Many of the problems concerned with closely related forms are much more complex. Ferris (31) described the situation in human lice and noted that the controversy, still unresolved, concerning the relationship of head and body lice began in 1761. He suggested that the taxonomic problem in human lice is as complex as, and comparable to, that presented by man himself. The butterflies of North America are generally believed to be very well-known taxonomically, but Klots (49) implied that they are not. For example, he wrote of *Colias* that some forms "appear to be in a very fluid evolutionary state, not having established definite entities or constant differences from others"; of *Pyrgus* that "in some cases we are not sure whether we are dealing with distinct species, subspecies, or very distinct varieties"; of *Lycaena phlaeas* (Linnaeus) that the "species" occurs from northern Ellesmere Island to the Gulf States. The lepidopterists can supply names for nearly all specimens of butterflies, hence the belief that the group has been well studied. But the relating of more or less similar

populations is too often based on insufficient evidence, intuition, or guesswork. According to Craighead (23), the spruce budworm [*Choristoneura fumiferana* (Clemens)], "one of the most destructive forest insects in North America," apparently consists of "a number of biological varieties, or races, that are distinct in their food habits." The spruce budworm of western North America is clearly a complex of very similar species that have not been, and apparently are not being, studied taxonomically [Freeman (34)]. Problems such as these will be resolved only by long and intensive study. But the task of the taxonomist is to examine them and to reexamine the accepted arrangements of closely related forms in the light of modern concepts and methods.

Concepts

Walsh (80, 81) was the first entomologist to consider the taxonomy of closely related species as a special problem. In 1863, four years after the publication of Darwin's *Origin of Species,* he tried to define the term "species" more objectively than had Darwin. He wrote:

> The only valid practical criterion of specific distinctness is the general nonexistence, either actually ascertained or analogically inferred, of intermediate grades in the distinctive characters, whence we may reasonably conclude that the two supposed species are distinct, i. e. that they do not now in general mix sexually together, or if geographically separated that they would not do so supposing them to be placed in juxtaposition.

He added that "the *amount* of difference between two species is comparatively nothing, the *constancy* of the difference is comparatively everything." Applying these concepts in 1864, he wrote at length on "phytophagic species," which he defined as phytophagous species that barely differ morphologically. Darwin (25) examined Walsh's concepts, rejected them, and continued to define the term "species" as one arbitrarily given for convenience to a group of similar individuals. Thus the local naturalist, impressed by the discreteness of species in nature, conflicted with the student of speciation, who was impressed by the evidence for common descent.

Systematists have reason to be bored with discussions of the species problem. Nevertheless the problem does exist, especially for the student of closely related species. In reviews of the problem, Mayr (55, 56) noted that all species criteria and all species definitions used in practical taxonomy are based ultimately on three basic concepts. These are the typological species concept, the nondimensional species concept, and the polytypic or multidimensional species concept. The definitions arising from these have one thing in common: they recognize the species as the breeding unit. In addition, forms that are obligatorily parthenogenetic, being fundamentally different from bisexual species, merit consideration as a special case.

The typological species concept.—This concept results in the morpho-

logically defined species, degree of morphological difference being the essential criterion. For example, Darwin (25) looked on "the term species as one arbitrarily given, for the sake of convenience, to a set of individuals closely resembling each other," and Thorpe (78) once defined the species as "a group of individuals distinguished from all other groups by the common possession of certain structural characters." The concept is unsatisfactory because it leads to a purely arbitrary definition. It requires the species to conform with an arbitrary, abstract, or ideal morphological pattern; variation is dealt with by arbitrarily fixed or by intuitive standards as to allowable deviation from the pattern. Thus specimens, not populations, are classified in the application of the concept [Simpson (72)]. This is the only concept that can be applied by the taxonomist working with a limited collection and with no knowledge of his species in nature. Most entomological taxonomy is based on this concept, of course, and much of it that deals with very similar forms is unsatisfactory on that account. The concept fails to deal adequately with intraspecific variation, especially with polymorphism, and with sibling, or cryptic, species, i.e., with discrete, reproductively isolated populations that lack obvious distinguishing characters. Taxonomists who are "lumpers" disagree with those who are "splitters"; the "species" of the former sometimes include several natural entities, and those of the latter are frequently not species in any meaningful sense. Taxonomic arrangements based on the concept often offer clues to undetected species; "difficult" groups are seen to need more study, and "species" that vary excessively or that show unusual distributional patterns are often composite. Ideally, the taxonomist dealing with very similar sympatric forms will not depend on the concept except when considering forms that are obligatorily parthenogenetic; most biologists who have given thought to the nature of species are agreed on this. Actually, most entomologists and many other biologists are satisfied with taxonomy based on this concept.

Rejection of the typological concept is not rejection of the notion that morphological criteria are useful in taxonomy. It is rejection of the idea that morphological characters can be evaluated arbitrarily to produce an objective taxonomy for closely related forms, for the morphological characters that distinguish species are of the same order as those that distinguish polymorphs and other variants within the species. The male genitalia, for example, are more useful than other body parts in separating insect species, yet in numerous groups they offer no characters for the separation of forms that are certainly species. In a few groups they are known to be extremely variable. Müller (62) showed that they are highly polymorphic in some species of the cicadellid genus *Euscelis*; the polymorphism is seasonal and is largely under photoperiodic control. Rupert (70) described seasonal dimorphism in the male genitalia of the geometrid genus *Plagodis*, and Brown (14) noted dimorphism in those of the chrysomelid genus

Chlamisus. Hubbell (45) described geographical variation in the male genitalia of grasshoppers.

The nondimensional species concept.—This concept is based on the relationship to one another of coexisting natural populations in a nondimensional system, i.e., at a single locality at one time. It is based not on differences between individual organisms but on the discreteness, due to reproductive isolation, of populations that are composed of freely interbreeding individuals. Absence of interbreeding between such populations, i.e., reproductive isolation, is the criterion. The criterion is nonarbitrary and results in a species definition that is objectively valid. Thorpe (79) defined the species as "a population of individuals prevented from interbreeding with all other populations by physiological differences (in the widest sense) whether or no structural differences are also present." Writing of *Drosophila,* Mainland (51) stated that one is dealing with two species "when the potentiality of gene interchange between two populations is reduced by positive isolating factors to a point less than their innate tendency to diverge." These statements define the nondimensional species. The concept recognizes the species as a group of individuals that share a common gene pool, and as an ecological unit that interacts as a unit with other species in the same environment. It can be applied only to organisms that reproduce bisexually; it does not deal with widely distributed species; and its application often requires knowledge, sometimes considerable, that can be acquired only by difficult techniques or study of organisms in their natural habitats. Nevertheless, because it is the only nonarbitrary concept, it is of utmost value to the understanding of sibling species and polymorphism and to the evaluation of taxonomic situations in general. The nondimensional species, being essentially an isolated gene pool, is usually but not necessarily distinctive morphologically; its morphological characters are a product of and are secondary to its genetic isolation. Thus the concept permits the segregation and recognition of species that apparently do not differ morphologically. All this being understood, it is legitimate and helpful to utilize morphological criteria in the application of the concept.

Many authors, including Dobzhansky (26), Mayr *et al.* (52, 53), Emerson (3), and Stebbins (73), have discussed the factors or mechanisms responsible for reproductive isolation. Isolating factors are usually classified as two types, extrinsic (spatial or geographical, in part) and intrinsic (reproductive or physiological). Extrinsic spatial isolation is that caused by natural barriers, the kind that has been overcome by the insects of foreign origin that are established in North America. The intrinsic factors are the ecological, which prevent the meeting of potential mates; the ethological, which cause males and females of different species to be unattractive to one another (sexual isolation); mechanical and physiological factors that prevent copulation and fertilization; and factors that cause varying degrees of inviability and sterility in hybrid offspring. As the species very rarely

cross-mate under natural conditions, sexual and ecological isolation are the effective factors in insects.

Breakdown of intrinsic isolating mechanisms, resulting in introgressive hybridization, i.e., the gradual infiltration of the germ plasm of one species into that of another, appears to be very rare in insects. The phenomenon, as it occurs in plants, has been described by Anderson (4). An apparent case of introgression in the leaf beetle genus *Chrysomela* is described below, and another in the gryllacridid genus *Ceutophilus* was described by Hubbell (45). According to Klots (49), the butterfly *Colias eurytheme* Boisduval has greatly extended its range in eastern United States during the past 35 years, where it hybridizes and competes with *Colias philodice* Latreille to the disadvantage of the latter. Hybrids have lowered fertility and survival value and form less than 1 per cent of the population in some districts but up to 10 per cent in others [Gerould (40)]. Hovanitz (43) presumed but did not demonstrate hybridization between several species of *Colias*. He believes that *Colias nastes* Boisduval and *Colias hecla* Lefebvre hydridize to produce *Colias boothii* Curtis, which is abundant in part of the region in which the others are sympatric. In other parts of their range, as at Churchill, Manitoba, *C. nastes* and *C. hecla* fly at the same time but occupy different habitats and do not hybridize. The evidence suggests that *C. boothii* is specifically distinct. Sympatric sibling species often show overlap in morphological characters and thus may simulate a complex of introgressing forms; the taxonomist should take care not to confuse two very different situations.

Many species of insects have been hybridized in the laboratory, and Patterson and Stone (65) reported the occurrence in nature of a small number of sterile males that resemble laboratory-produced hybrids of *Drosophila mulleri* Sturtevant and *Drosophila aldrichi* Patterson & Crow. Patterson (64) attempted 110 interspecific crosses with 11 species of the *tripunctata* group of *Drosophila* but obtained matings in only 13 cases. Progeny resulted from seven of the crosses and showed varying degrees of viability; in three cases the hybrids were interfertile. Patterson and Stone (65) described a large number of interspecific crosses in *Drosophila*. Progeny of successful crosses showed greatly varying degrees of viability and fertility. Such work is of interest to the student of natural populations because it illustrates the great importance of sexual isolation and also the futility of attempts to base a species concept on hybrid sterility or lack of it. Writing of vertebrates, Blair (10) stated: "It seems to be generally true that diverging populations of animals usually acquire mechanisms of reproductive isolation before they have diverged genetically to the point of intersterility." He concluded that "hybrids between sympatric species of vertebrates are rare, and they usually occur where there has been a breakdown of ecological isolation," and that "the amount of gene transfer between sympatric species of vertebrates is unknown, but it seems

probable that it is negligible." Blair's conclusions appear to apply well to insects.

The term "sibling species" was proposed by Mayr (52, 53) merely as a convenient term to designate those species that are morphologically very similar or indistinguishable. Such species also have been designated "cryptic," "physiological," and "phytophagic" species. They have been discussed by many authors, including Dobzhansky (26), Mayr (53), Patterson & Stone (65), and White (84). Sibling species are like other species except that they are more difficult to segregate. They are not necessarily sympatric, but they are detected most commonly when they coexist. They have been revealed by differences in ecological relationships, behavior, and chromosome morphology. Their validity as species cannot be questioned if reproductive isolation is accepted as the essential criterion of the nondimensional species. Some authors maintain that physiological or biological races occur sympatrically in insects. For example, Thorpe (77, 78, 79) stated that biological races "exist where the individuals of a species can be divided into groups, occurring in the same locality and showing definite differences in biology, but with structural differences, either very slight and inconstant, or completely absent." He treated as biological races sympatric forms that resemble one another morphologically and occur in different ecological niches. However, such forms can exist as natural populations only if gene flow between them is prevented, in which case they qualify as species. Thorpe accepted the typological species concept. It follows that the sympatric physiological or biological races of the typological species concept are sibling species in the nondimensional concept.

The polytypic or multidimensional species concept.—This concept considers the species a group of populations that are actually or potentially capable of interbreeding. It recognizes the fact that species are distributed in space and time, and it is essentially the nondimensional concept extended to cover those factors. Thus Mayr (52, 53, 56) defined species as "groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups." Dobzhansky (26) defined the species as "the most inclusive Mendelian population," the Mendelian population being the breeding population. Inclusion of the space factor creates difficulty in application of the concept, the difficulty of relating properly more or less similar allopatric forms. This difficulty gives both working taxonomists and theorists their greatest problem and greatest area of disagreement. The important criterion, the presence or absence of isolating mechanisms, cannot be applied to allopatric forms, not even experimentally in some cases, for some forms will not breed in confinement, and those that do hybridize successfully in the laboratory might be isolated by ecological or ethological factors if they should come together in nature. Furthermore, forms that must be considered geographical variants of one species may not hybridize successfully. Hubbell (45) found great

clinal variation in the male genitalia of the grasshopper *Melanoplus puer* (Scudder) and suspected that insemination of females from one end of the species' range by males from the other end would be impossible. Moore (61) demonstrated that variation in temperature tolerance and rate of development is clinal in the embryo of the frog *Rana pipiens* Schreber; hybrids between northern and southern populations are inviable. Because populations are adapted by natural selection to the regions in which they live, physiological clines such as those in *Rana* are to be expected in some widely distributed species. Dobzhansky (26) pointed out that natural selection may be expected to maintain and perfect the reproductive isolation of sympatric species. Closely allied allopatric forms, not being subject to such selection, lack this perfecting mechanism and on that account may show reduced isolation if brought together, as compared with comparable sympatric forms. Thus the criterion of reproductive isolation may tend to fail when applied to allopatric forms. It follows from all this that the multidimensional species lacks the objective validity of the nondimensional species.

To emphasize the inherently subjective nature of the taxonomy of allopatric forms, Mayr (52) stated:

> . . . every one of the lower systematic categories grades without a break into the next one: the local population into the subspecies, the subspecies into the monotypic species, the monotypic species into the polytypic species, the polytypic species into the superspecies. . . . This does not mean that we can find the entire graded series within every species group. It simply means that in the absence of definite criteria it is, in many cases, equally justifiable to consider certain isolated forms as subspecies or as species, to consider a variable species monotypic or to subdivide it into two or more geographical races, to consider well-characterized forms as subspecies of a polytypic species or to call them representative species.

The difficulties produced by this situation are obvious, and there can be no completely satisfactory method of dealing with them. The problem, for our purposes, centers in the nature and definition of the subspecies, which most taxonomists equate with the geographical race.

Mayr, Linsley & Usinger (57) defined subspecies as "geographically defined aggregates of local populations which differ taxonomically from other such subdivisions of a species." The meaning of the definition depends on the interpretation of the phrase "differ taxonomically," and taxonomists may disagree on how comprehensive this should be. Population geneticists have shown that no two natural populations are genetically identical and that a single population will vary genetically over a period of time. Hence the subspecies, like the species, is an assemblage of differing populations, except extremely localized relict or insular subspecies. Mayr *et al.* (57) recommended that the status, species or subspecies, of differing allopatric forms be decided by inference. The forms may be treated as species if their differences are of the order that commonly distinguishes sympatric species of the genus, or as subspecies if their char-

acters are of the type known to vary geographically within species of the genus. They recommended that allopatric forms be treated as subspecies, rather than as species, in doubtful cases, because use of the trinomial has the practical advantage of indicating both closest relationship and allopatry. Mayr (53) noted that the method leads to recognition of subspecies of very different value, the range extending from the poorly defined intergrading population to the well-defined, isolated, allopatric form, but that the species concept thereby gains in conciseness what the subspecies concept loses. Most taxonomists agree well or fairly well with Mayr *et al.* and attempt to follow the definition and methods of which they approve.

Wilson & Brown (86) concluded "that the subspecies concept is the most critical and disorderly area of modern systematic theory," and that the subspecies "has a demonstrably flimsy conceptual basis." They accepted Mayr's statement, quoted above, that the lower systematic categories grade into one another. They stated:

> Mayr's criterion of the species, that of free interbreeding of populations in nature, when qualified by conditions of sympatry and synchrony, and extended by morphological analogy to isolated populations, has proved to be objective and practicable for taxonomic work.

They attributed the difficulties of delimiting the subspecies to four features of geographical variation. (*a*) Genetically independent characters vary independently, and their geographical variation tends to be discordant to some degree, i.e., a species may increase gradually in size from north to south but may change gradually in color from east to west. The greater the number of characters that vary, the greater may be the total discordance. Most taxonomic recognition of subspecies assumes that characters tend to be concordant in their geographical variation. Taxonomists may delimit subspecies on one or several obvious characters, ignoring other geographically variable characters or analyzing them only in terms of the subspecific units previously defined. Or taxonomists may choose several discordant characters, employ them in combinations of two or three to establish racial limits, and then analyze each character individually in terms of those limits. The result may be the recognition of unnatural "subspecies." (*b*) If a geographical race is delimited by a single character, the character may be selected to predominance in more than one region to produce a polytopic race. (*c*) Microgeographical races occur commonly; all widespread species tend to form local populations with relatively homogeneous and more or less distinctive genetic constitutions. (*d*) Even when there apparently is no discordant variation, there is no real or satisfactory lower limit to the subspecies category. Concerning forms that apparently are well-characterized insular races, Wilson & Brown found that the possibility of specific distinctness has seldom been eliminated. They concluded that the subspecies trinomial, as a formal means of registering geographical variation within the species, tends to be both illusory and superfluous. They stressed an important and frequently neglected point, that genetically

independent characters often vary discordantly, a fact to be considered if subspecies are to be entities in any meaningful sense. Mayr (54) stated that critics of the subspecies concept

> . . . are quite right in insisting on the subjective nature of the subspecies and in criticizing the excessive splitting of continuous, clinal chains of populations, but that attempts to eliminate the subspecies category altogether are by no means an improvement on the situation.

This means in part that segments of a continuous, clinal chain may not "differ taxonomically." Eliminating the subspecies as a category does not lessen the difficulties in relating allopatric forms. Wilson (85) demonstrated this in the ant genus *Lasius*. He found, for example, in *Lasius alienus* (Foerster) that the North American populations appear "to be separable as a unit" from the Eurasian on the basis of male size, and that the North American and Eurasian populations "differ markedly from one another in habitat preference," the ants inhabiting well-shaded woodland in the former region and open, dry situations in the latter. He believed that in both regions "*alienus* has been squeezed into relatively narrow ecological ranges by its congeneric competitors," and that the ants have evolved and diverged accordingly. Unlike Wilson, most taxonomists would consider the populations of the two regions subspecifically distinct, or specifically distinct because sympatric sibling species are numerous in the genus.

Durrant (29), writing of mammals, defended the subspecies, and its trinomial, as an important tool in the study of geographical variation and distribution, because it indicates relationships, degrees of difference, and the significance of variation. He viewed the subspecies as one or more populations that have attained sufficient genetic stability and distinctness to develop a recognizable phenotype in the majority of its members. He found that recognition of such subspecies establishes standards for meaningful studies of variation, standards especially useful in the study of mammals of mountainous regions. He noted that "subspecies" and "cline" are terms with different meanings and that the subspecies concept may be inadequate or unnecessary in the study of mammals of the Great Plains because of their extensive ranges and wide zones of intergradation. The subspecies of Durrant are more or less insular natural entities that differ from species only in degree of genetic isolation.

Hubbell (44) stated: "There appears to be far more diversity among insects than among vertebrates in the factors that may produce isolates within species, and the entomologist may consequently have to deal with a greater variety of resultant taxonomic situations." Concerning insects, he concluded "that population analysis below the species level is too complex to be bound by formal taxonomic and nomenclatorial rules, and that each case must be considered on its own merits and treated by whatever method seems best suited to it." He asserted that: as clinal variation is variation of characters in population, clines themselves cannot be taxo-

nomic units; in a species showing only a stepped cline in a single character or in a group of correlated characters, it is feasible and may be convenient to treat the segments between steps as subspecies; in species in which clinal change is gradual or in which two or more discordant clines exist, it is inadvisable to recognize subspecies. In another paper Hubbell (45) described and discussed types of geographical variation that occur in insects.

Parthenogenetic forms.—A periodic occurrence of parthenogenesis, as in aphids, or facultative parthenogenesis does not alter the essential nature of the species, which remains a group of populations that are actually or potentially able to exchange genes. But when parthenogenesis is obligatory, there is no gene flow except within each family line, and an entirely different kind of species results. Although the taxonomy of obligatorily parthenogenetic forms must be arbitrary, it can be meaningful. Examples in the weevil subfamily Brachyrhininae (=Otiorhynchinae) illustrate situations that arise. Suomalainen (75, 76) showed that European parthenogenetic forms of the group are usually polyploid. One or more parthenogenetic forms may resemble morphologically a diploid, bisexual form, which usually has a more southern distribution and which is presumed to be ancestral to the parthenogenetic forms. For convenience, such forms are treated as races of one species. For example, *Otiorhynchus scaber* (Linnaeus) occurs as a triploid parthenogenetic race in the Austrian Alps, as a tetraploid parthenogenetic race in Finland, and as a bisexual race in the eastern Alps. Some European parthenogenetic forms are rather similar to but separable from more southern bisexual forms and are, for convenience, treated as specifically distinct from the latter. Other parthenogenetic forms are well-characterized morphologically and are treated as species. Sometimes parthenogenetic forms are more difficult to arrange. In the brachyrhinine genus *Graphognathus,* Buchanan (19) found that some of them "exhibit exceedingly close and peculiarly involved relationships," and that "statistical study of long series indicates that *fecundus* and *imitator* are separate entities, even though they merge more or less imperceptibly in all characters and character combinations." Buchanan arbitrarily treated the well-characterized forms as species and the allopatric intergrading forms, such as those mentioned, as races of the "species" they most closely resemble. Situations like that described by Buchanan are common in asexual organisms, but most obligatorily parthenogenetic insects are as well-characterized as their bisexual allies and may be treated accordingly taxonomically. White (82) concluded that "in most parthenogenetic coccids, weevils, sawflies and stick insects we can speak of 'species' without impropriety, since many of the specific characters are probably older than the asexual method of reproduction, and have been handed down from the time when these forms were bisexual." The evolution and significance of parthenogenesis have been thoroughly reviewed by White (82, 83).

Regarding species concepts in general, it is well to remember that several may be applied, even in a single taxonomic paper, in the study of closely related forms. Some concepts lead to conclusions that are subjective or tentative, others to conclusions that are objectively valid, but objectively valid species may have very different properties. The simple binomial or trinomial nomenclature cannot depict the great variety of situation that taxonomists perceive, and taxonomists perceive only the more obvious of the situations. Consequently the best taxonomic arrangements are greatly oversimplified.

Methods

The methods required to segregate very similar forms result naturally from the concepts adopted by the taxonomist. Ideally taxonomic studies of closely related forms are based primarily on the nondimensional species, because they are the only objectively valid species. Species have three attributes that are taxonomically important; they differ physiologically and morphologically, and they occur as discrete entities in nature. Physiological differences between species are reflected in ecological relationships and behavior and are revealed by study of populations in nature. Morphological differences between species are, of course, revealed by study of preserved samples. Even sibling species usually differ morphologically to some degree in some stage. Ideally the specimen of each population preserved for study is a random sample; ideally each sample includes all stages and is comprehensive enough to show the range of variation and the frequencies of the variants within the population. Special techniques, as well as direct observation, may be employed to determine the discreteness of forms that may or may not be specifically distinct. After the species of a region have been studied and compared, the taxonomist is prepared to relate them to the more or less similar forms of other regions.

In practice the taxonomist begins by becoming dissatisfied with the accepted arrangement of the species of a group. He may find the arrangement based on unsatisfactory concepts, methods, or materials. Or the arrangement may recognize "species" that show unnatural distributional patterns or that vary excessively in morphology, behavior, or ecological relationships. Ordinary insect collections often reveal the need for special studies but are usually useless as an aid in segregating sibling species. To understand these, the taxonomist requires special collections and knowledge of populations in their natural habitats. His field work is designed to relate properly to one another the populations of one region and to trace distribution and geographical variation from one region to another. After he has segregated sibling species by ecological or ethological characters, he can often find obscure morphological characters to separate them. Mayr, Linsley & Usinger (57) offer a comprehensive treatment of taxonomic characters and of methods of evaluating them. Methods of segregating closely related forms are suggested by examples described below.

Orthoptera.—In this as in other orders, there is great variation within some species and an abundance of sibling species, a situation that makes evaluation of morphological characters difficult. The great variation related to density of nymphs in gregarious species of locusts is well known [Key (48)]. Hubbell (45) described types of geographical variation shown by North American grasshoppers. He found that *Melanoplus puer* (Scudder), a flightless species of Florida, occurs as a large number of microgeographical races; 22 of the 27 local populations observed in central Florida could be distinguished from all others by genital characters. He described linear, centripetal, and stepped clines in other grasshoppers. In *Aptenopedes sphenarioides* Scudder, he found an extremely complex situation caused by several discordant clines and other geographical variation. Breland & Dobson (11) found evidence that the mantids now recognized as *Stagmomantis limbata* (Hahn) should be considered two species on the basis of differences in oothecae, nymphs, and distribution. Alexander (2) estimated that 10 per cent of the species of Tettigoniidae and Gryllidae of the eastern United States are unrecognized sibling species.

Some of the sibling gryllids have been studied. In 1908 Lutz (50) studied variation in length of tegmina, wings, and ovipositors in a large number of specimens of *Acheta* (=*Gryllus*) from many regions. He concluded that "species in an any way natural sense do not exist in the genus." In 1915 Rehn & Hebard (66) reached a similar conclusion but found "that in general certain types do predominate over certain regions." In 1952 Fulton (38) segregated four "races or populations" of *Acheta* in North Carolina on the basis of differences in song, seasonal history, and habitat; all of 50 attempts to hyridize the different forms failed, although pairs of the same form rarely failed to produce offspring. Alexander (2) extended Fulton's study. He found an additional sibling and showed that at least three of the five siblings are widely distributed in the eastern United States. He found morphological characters to separate all but one pair of the species in at least one sex. He attempted 30 interspecific crosses and obtained numerous matings; only three crosses produced hybrids, and he concluded that these "probably do not reflect a lack of reproductive isolation in the field." Fulton (36) found the situation in *Nemobius* exactly like that in *Acheta,* and he (35, 37, 39) found but did not study similar situations in the gryllid genera *Oecanthus, Anaxipha,* and *Cycloptilum,* and in the tettigoniid genus *Amblycorypha.*

Some species of the order have been segregated by differences in their chromosomes. The grasshopper *Mermiria maculipennis* Bruner shows striking clinal change from north to south; the northern populations were confused with another species until cytological evidence revealed the proper relationship [Hubbell (44)]. White (84) stated that certain allopatric species of morabine grasshoppers would almost certainly be regarded as subspecies in the absence of cytological evidence. He showed by cytological evidence that presumed subspecies of the grasshopper *Austroicetes pusilla*

(Walker) are actually two species which overlap in their morphological characters; clinal variation in one species or introgressive hybridization between two species is simulated. He noted also instances in which presumed species of crickets and mole crickets appear to be divisible into sibling forms, species or subspecies, on cytological characters.

Homoptera.—Ross (68) noted that insects of different species sometimes appear to live their entire lives in identical situations. He studied six very closely related species of the cicadellid genus *Erythroneura* that live on the sycamore, *Platanus occidentalis* Linnaeus, and that have identical life histories and habits. He concluded that many insect species can share such habits without competing with one another. This is in contradiction to Gause's rule, which holds that species with identical ecological requirements cannot coexist. The matter is of interest to taxonomists because of the taxonomic importance of ecological relationships. In cases such as Ross described, the taxonomist must determine whether the forms concerned are different species or polymorphs of one species.

There is evidence of considerable host specificity in the Homoptera, but the food relationships have received inadequate study and have been neglected by taxonomists. Writing of cicadellids, Oman (63) emphasized the need to distinguish between host plants, on which eggs and nymphs occur, and food plants, which adults use for food but not for oviposition. The same distinction is important in many groups, especially in the spring, when most plants are succulent, and in late summer, when host plants may be unsuitable for feeding.

Coleoptera.—There is abundant evidence of sibling species in this order, especially in the Rhynchophora and Chrysomelidae. *Pissodes, Dendroctonus,* and *Ips* are examples of rhynchophoran genera that require much study. Larvae of the curculionid *Hylobius radicis* Buchanan feed on the root crowns of living young pines, and those of *Hylobius pales* Herbst feed beneath the bark of pine logs and stumps. This difference in habit was the clue that caused Buchanan (18) to suspect that two species were confused and to seek and discover the feeble morphological characters that distinguish the adults. In the chrysomelid genera that feed on leaves in the larval stage, as do those mentioned below, there is a high degree of host specificity. This furnishes the only known basis for segregating some of the species, although slight, often inconstant morphological differences can usually be detected after the species have been distinguished.

The American forms identified commonly as *Chrysomela interrupta* Fabricius are a complex of siblings [Brown (17)]. Morphologically they appear identical except for differences, inconstant and usually slight, in color and size. Adults vary greatly both within populations and geographically, and there is much dichromatism in eggs, larvae, and adults. Three of the northern forms feed and breed on *Alnus.* One of these ranges from Newfoundland to the Rocky Mountains. It is replaced to the west

by a form that averages larger and darker; this form, in turn, is replaced west of the Coast Ranges by one that averages larger and darker still. The three appear to be strictly allopatric because of the mountain barriers. Although their characters are like those that distinguish sympatric species, they are presumed to be only subspecifically distinct, because, as noted below, sexual isolation appears to be weak in the group and the sympatric siblings that have been tested hybridize freely in the laboratory. In the Kenai Peninsula, Alaska, extremely variable populations were found on *Salix* and *Populus*. Beetles from the two hosts are presumed to be of one species because: collections of all stages, taken from the two hosts where they were intermixed, showed the same range of variation and the same frequencies of the variants; when hosts were intermixed, the beetles always occurred in similar numbers on both; larvae and adults showed no preference for either host in the laboratory. Seasonal history was the same on the two hosts, but it is usually the same in all species of this genus at one locality. Also in the Kenai Peninsula, a colony of beetles was found on *Alnus*. Adults from this colony resembled many of those from the other hosts, but most of the mature larvae from *Alnus* differed in color from those of the others. Newly hatched larvae of the *Alnus* form were reared with moderate mortality on *Populus*, but larvae of the *Salix-Populus* form did not develop satisfactorily on *Alnus*. Two species are indicated. With some geographical variation, the *Salix-Populus* species extends to Labrador and Newfoundland. At Gander, Newfoundland, it occurred with the eastern *Alnus*-feeder where the hosts were intermixed. Adults of these species differ strikingly in color, and adults of each were observed only on the normal host. Newly hatched larvae of both were reared without mortality on the parental hosts, but larvae of neither could be reared on the host of the other. The two are obviously distinct. When males of the *Salix-Populus* species were confined with females of the *Alnus* species at Gander, the beetles copulated freely and produced vigorous hybrids; hybrid larvae survived well on both hosts to produce adults that were intermediate in color between the parent species. In easternmost Ontario, this *Alnus*-feeder occurs with a rare form that averages darker and slightly larger in the adult stage, and which is restricted to *Populus balsamifera* Linnaeus. Some adults from the two hosts cannot be distinguished, and the immature stages are inseparable. Newly hatched larvae of both forms were reared with moderate mortality on the parental hosts, but larvae of the *Alnus*-feeder fed very briefly and died without growing when confined on *Populus*. When confined on *Alnus*, larvae of the *Populus*-feeder usually died without growing, but 24 of the 65 egg masses tested produced a few larvae that tolerated *Alnus* to varying degrees, and five of these masses produced a few adults. Two species are indicated. When males of each species were confined with females of both, the beetles copulated freely and showed no species discrimination. Reciprocal crosses produced vigorous hybrids, which survived well on

both hosts. In southernmost Ontario, a single colony of beetles was found on *P. balsamifera.* This colony is presumed to be of the same species as the *Populus*-feeder if eastern Ontario, although it differs in having some of the eggs and adults of a different color and similar to those of a *Salix*-feeder, which is the common species of that region. This *Salix*-feeder does not breed on *Populus* but feeds on it in early spring, and it was once found copulating freely with a congeneric species to which it is not closely allied. Possibly it invaded the colony on *Populus,* and introgressive hybridization resulted. And possibly the ability of some larvae of the *Populus* species to feed on *Alnus* indicates introgression with the *Alnus* species in easternmost Ontario. To understand the species of such groups of variable siblings, the taxonomist must compare and relate the sympatric populations of several localities by field and laboratory studies and must trace distributional patterns and geographical variation in order to relate populations of different regions.

The situation in the chrysomeline genus *Calligrapha* is rather similar to that in *Chrysomela* [Brown (15)]. In eastern Ontario, breeding colonies occur sympatrically on *Ulmus americana* Linnaeus, *Tilia americana* Linnaeus, and *Ostrya virginiana* (Miller) K. Koch. Adults from the three hosts are barely distinguishable by differences in color pattern, and their larvae differ only by overlapping characters in maculation. Except for the *Ulmus*-feeder, which survives well on *Tilia,* newly hatched larvae of none of these siblings attempt to feed when confined on the hosts of the others. Two sympatric species of southern Ontario, one restricted to *T. americana,* the other to *Prunus americana* Marshall, differ as adults only in average size and by a very slight difference in body form, but larvae of the two differ strikingly in color.

A perplexing situation occurs in the chrysomelid genera *Chlamisus* (=*Chlamys, Arthrochlamys*) and *Lema.* In easternmost Ontario, the sibling species of *Chlamisus* depend on different hosts and show feeble morphological characters like those of *Calligrapha* [Brown (13)]. However, populations that are restricted to one species of *Salix* in easternmost Ontario, to *Corylus* in southernmost Ontario, and to *Alnus* in Nova Scotia appear to be inseparable [Brown (16)]. Forms apparently morphologically identical, all considered *Lema trilineata* (Olivier), breed on *Physalis* and potato in southernmost Ontario and the eastern United States but on *Physalis* exclusively in easternmost Ontario and in Manitoba. Criddle (24) was unable to maintain caged populations on potato in Manitoba. In these examples, either the species vary geographically in feeding habits, or sibling species are confused. When very abundant, some of the Chrysomelinae expand their feeding to include species closely related to their normal hosts. *Phratora purpurea* Brown is normally restricted in Ontario to *Populus tremuloides* Michaux; during a season of unusual abundance, it bred without evident preference on all native species of *Populus* and on

several species of *Salix*. Similar examples have been noted in *Chrysomela* and *Gonioctena*.

Diptera.—In this order, cytology has been a very useful taxonomic tool. Patterson & Stone (65) described cytotaxonomic characters of approximately 200 species of *Drosophila*. They found that members of the same species group often have very different karyotypes. According to White (84), all cytological differences between species, however minute, in theory should be observable in those Diptera that possess giant chromosomes in the salivary glands. And in theory, all species of insects should differ cytologically. Gene sequences, which are undetectable in other organisms, are readily observed in the giant chromosomes of the Diptera. Dunbar (28) studied the salivary chromosomes in the black fly genus *Eusimulium*. He segregated sibling species, previously undetected, by arrangements within, and polymorphism in, the chromosomes. Carson (21) distinguished three siblings in *Drosophila* by inversions in the salivary chromosomes. Two of these are sympatric and indistinguishable morphologically; they apparently do not hybridize in nature, although they produce vigorous, fertile hybrids in the laboratory. Salivary chromosomes have been studied in *Anopheles, Sciara,* and *Chironomus,* in several groups of *Drosophila,* and in the Itonididae, Simuliidae, and Agromyzidae, but they offer a field that has scarcely been touched.

Barnes and his colleagues have shown that the host relationships of itonidid midges afford useful taxonomic characters. For example, Stokes (74) demonstrated two species of *Dasyneura,* apparently identical morphologically, that require as hosts different species of *Viola*. The situations that arise in these midges were described and discussed by Barnes (6). Host ranges of the midges are determined by tests in cages; in such studies, it is well to seek corroborative evidence in natural populations, because caged material may behave abnormally.

The occurrence of sibling species in the mosquito genera *Anopheles* and *Culex* has been known for many years [Bates (7)]. Knowledge of the siblings has developed slowly, partly because they present complex problems, and partly because of the inadequate concepts and methods applied to their study. Much recent work seems designed to explore or to rediscover problems, rather than to contribute to solutions. There are numerous examples in the studies described by Rozeboom & Kitzmiller (69). The needs, especially in *Culex,* seem to be: greater emphasis on comparative studies of sympatric populations and on the nature of geographical variation in order to provide knowledge of sympatric species and a basis for relating allopatric forms; less emphasis on comparisons of allopatric forms until a better basis for comparisons has been established; an adequate species concept. Regarding concepts recently applied, Rozeboom & Kitzmiller (69) stated: "Populations separated by genetic or other barriers which would restrict gene flow are considered to be specifically distinct;

populations differing from one another by some morphological or biological character, but capable of hybridization, are recognized as subspecies." The first part of this statement can be applied to delimit objectively valid species. The second part conflicts with the first part and affords no basis for a meaningful concept of either the species or the subspecies. Sibling species are known in other genera of mosquitoes. According to Carpenter & La Casse (20), adults of the three North American species of *Orthopodomyia* are scarcely distinguishable, but the larvae are strongly characterized and readily distinguished.

Various degrees of sexual isolation between laboratory populations of *Drosophila,* presumably of one species but from different regions, have been reported by several authors [Miller (60)]. The same phenomenon has been reported in mosquitoes [Rozeboom & Kitzmiller (69)]. Studies are not detailed enough to show the geographical pattern of such isolation in any species. The situation affords another example of the difficulties in relating properly more or less similar allopatric forms.

Hymenoptera.—The aculeate Hymenoptera, wih their complex behavior patterns, offer a rich field for studies in comparative ethology. Evans (30) discussed the taxonomic value of ethological characters and noted examples in which behavior provided the basis for separating sibling species. The digger wasps that have been identified as *Ammophila campestris* Latreille provide an outstanding example. Adriaanse (1), studying behavior, discovered that two siblings were confused. They differ in methods of opening, closing, and provisioning the burrows and in seasonal history; one species provides its larvae with caterpillars, the other with sawfly larvae.

Atwood & Peck (5) segregated pine-feeding siblings of the sawfly genus *Neodiprion* by differences in seasonal history, egg-laying habits, and color of larvae and cocoons, which they correlated with slight differences in the ovipositors. Benson (8) noted that most British sawflies are highly restrictive in their feeding, and that "apparent polyphagy often hides a subdivision of the species into highly specialized foodplant races." As "foodplant races," he cited among others *Pristiphora quercus* (Hartig), which feeds on *Betula* and *Vaccinium* but not on other plants, and *Nematus vagus* (Fabricius), which depends on *Salix* and *Carex*. It is almost certain that the so-called races are sibling species.

Flanders (32), writing of chalcidoids, noted that the host specificity of a parasite in one region may not be the same as the specificity in another. He cited several examples of presumed host-limited geographical races, including *Comperiella bifasciata* Howard, the Japanese and Chinese populations of which require as hosts different species of the coccid genus *Aonidiella.* The situation appears comparable to that described above in the chrysomelid genera *Chlamisus* and *Lema.*

Lepidoptera.—Problems in this order, as previously suggested, concern the segregation of sibling species and the relating of more or less similar allopatric forms. Graham (41) noted in Minnesota that the spruce bud-

worm, *"Archips fumiferana,"* defoliated balsam fir but avoided jack pine in one area, and defoliated jack pine but avoided the other host in another region. He found by observation and laboratory tests that the adult moths show a decided preference for the host on which they were reared, and he concluded that "we are justified in treating the two forms in forest management as if they were two distinct species." It is now known that the spruce budworm is a complex of siblings and that Graham's forms are distinct species [Freeman (34)]. Graham interpreted his observations according to Hopkins' host selection principle, which holds that the breeding of a polyphagous species on one species of host for a few generations may produce a strain that prefers or even requires that host [Craighead (*22*)]. The principle cannot be considered valid, because it depends on ideas that now appear unsound. Brown (12) described butterflies of the satyrid genus *Coenonympha.* He compared statistically population samples of the forms, which vary considerably both clinally and within populations. He described distributional patterns and ecological relationships. Thus he produced a picture of the forms as they occur in nature. He offered a taxonomic arrangement, which was necessarily and admittedly arbitrary. However, the picture presented permits independent interpretation, and the work thus avoids the dogmatism that mars some treatments of similar taxonomic situations. Mayr, Linsley & Usinger (*57*) described statistical methods that are useful in taxonomy.

Biochemical methods.—Recently attempts have been made in taxonomic studies to consider differences in protein composition. The methods present some difficulties when applied to insects, and it is too early to judge their usefulness as tools in the study of closely related species. Downe (*27*) described briefly the application of serological methods to the study of insects. Micks (58) and Micks & Gibson (59) discussed the application of paper chromatography to insect taxonomy. Zweig & Crenshaw (*87*) studied serum proteins of the turtle genus *Pseudemys* by paper electrophoresis. They were able to separate species but not subspecies and concluded that the technique may be employed effectively in intrageneric comparisons of some animal groups.

LITERATURE CITED

1. Adriaanse, A., *Behaviour*, **1**, 1–34 (1947)
2. Alexander, R. D., *Ann. Entomol. Soc. Am.*, **50**, 584–602 (1957)
3. Allee, W. C., Emerson, A. E., Park, O., Park, T., and Schmidt, K. P., *Principles of Animal Ecology* (W. B. Saunders Co., Philadelphia, Pa., and London, England, 837 pp., 1949)
4. Anderson, E., *Introgressive Hybridization* (John Wiley & Sons, Inc., New York, N. Y., 109 pp., 1949)
5. Atwood, C. E., and Peck, O., *Can. J. Research*, **21**, 109–44 (1943)
6. Barnes, H. F., *Ann. Entomol. Fennici*, **19**, 2–24 (1953)
7. Bates, M., *The Natural History of Mosquitoes* (The Macmillan Co., New York, N. Y., 379 pp., 1949)

8. Benson, R. B., *Trans. Soc. Brit. Entomol.*, **10,** 45–142 (1950)
9. Birch, L. C., *Australian J. Exptl. Biol. Med. Sci.*, **22,** 271–75 (1944)
10. Blair, F., *Am. Naturalist*, **85,** 9–30 (1951)
11. Breland, O. P., and Dobson, J. W., *Ann. Entomol. Soc. Am.*, **40,** 557–75 (1947)
12. Brown, F. M., *Bull. Am. Museum Nat. Hist.*, **105,** 363–409 (1955)
13. Brown, W. J., *Can. Entomologist*, **75,** 119–31 (1943)
14. Brown, W. J., *Can. Entomologist*, **76,** 70–72 (1944)
15. Brown, W. J., *Can. Entomologist*, **77,** 117–33 (1945)
16. Brown, W. J., *Can. Entomologist*, **84,** 335–42 (1952)
17. Brown, W. J., *The New World Species of* Chrysomela *L.* (*Can. Entomologist*, **88,** Suppl. 3, 54 pp., 1956)
18. Buchanan, L. L., *Proc. Entomol. Soc. Wash.*, **36,** 252–56 (1934)
19. Buchanan, L. L., *J. Wash. Acad. Sci.*, **37,** 19–22 (1947)
20. Carpenter, S. J., and La Casse, W. J., *Mosquitoes of North America* (University of California Press, Berkeley and Los Angeles, Calif., 360 pp., 1955)
21. Carson, H. L., *Evolution*, **8,** 148–65 (1953)
22. Craighead, F. C., *J. Agr. Research*, **22,** 189–220 (1921)
23. Craighead, F. C., *Insect Enemies of Eastern Forests* (*U. S. Dept. Agr., Misc. Publ. 657*, 679 pp., 1950)
24. Criddle, N., *Can. Entomologist*, **65,** 150–51 (1933)
25. Darwin, C., *The Origin of Species by Means of Natural Selection*, 6th ed. (John Murray, London, England, 458 pp., 1882)
26. Dobzhansky, T., *Genetics and the Origin of Species*, 3rd ed. (Columbia University Press, New York, N. Y., 364 pp., 1951)
27. Downe, A. E. R., *Rutgers Univ. Serol. Museum Bull.*, **19,** 3–5 (1957)
28. Dunbar, R. W., *Can. J. Zool.*, **36,** 23–44 (1958)
29. Durrant, S. D., *Systematic Zool.*, **4,** 186–90 (1955)
30. Evans, H. E., *Systematic Zool.*, **2,** 155–72 (1953)
31. Ferris, G. F., "The Sucking Lice" in *Memoirs of the Pacific Coast Entomological Soc.*, **1** (San Francisco, Calif., 320 pp., 1951)
32. Flanders, S. E., *J. Econ. Entomol.*, **43,** 719–20 (1950)
33. Floyd, E. H., and Newsom, L. D., *Two Species of Rice Weevils in the United States* (Presented at Entomol. Soc. Amer., 5th Meeting, Memphis, Tenn., 1957)
34. Freeman, T. N., *The Archipinae of North America* (*Can. Entomologist*, Suppl. 7, 89 pp., 1958)
35. Fulton, B. B., *Ann. Entomol. Soc. Am.*, **18,** 363–83 (1925)
36. Fulton, B. B., *Ann. Entomol. Soc. Am.*, **24,** 205–37 (1931)
37. Fulton, B. B., *Ann. Entomol. Soc. Am.*, **26,** 368–76 (1933)
38. Fulton, B. B., *Evolution*, **6,** 283–95 (1952)
39. Fulton, B. B., *J. Elisha Mitchell Sci. Soc.*, **72,** 222–43 (1956)
40. Gerould, J. H., *Ann. Entomol. Soc. Am.* **39,** 383–95 (1946)
41. Graham, S. A., *Papers Mich. Acad. Sci.*, **9,** 517–23 (1929)
42. Gurney, A. B., and Brooks, A. R., *Proc. U. S. Natl. Museum* (In press)
43. Hovanitz, W., "Increased Variability in Populations Following Natural Hybridization," in *Genetics, Paleontology, and Evolution*, 339-55 (Jepsen, G. L., Mayr, E., and Simpson, G. G., Eds., Princeton University Press, Princeton, N. J., 474 pp., 1949)
44. Hubbell, T. H., *Systematic Zool.*, **3,** 113–21 (1954)

45. Hubbell, T. H., *Ann. Rev. Entomol.*, **1**, 71–88 (1956)
46. Huxley, J., Ed., *The New Systematics* (Oxford University Press, London, England, 583 pp., 1940)
47. Jepsen, G. L., Mayr, E., and Simpson, G. G., Eds., *Genetics, Paleontology, and Evolution* (Princeton University Press, Princeton, N. J., 474 pp., 1949)
48. Key, K. H. L., *Quart. Rev. Biol.*, **25**, 363–407 (1950)
49. Klots, A. B., *A Field Guide to the Butterflies of North America, East of the Great Plains* (Houghton Mifflin Co., Boston, Mass., 349 pp., 1951)
50. Lutz, F. E., *Carnegie Inst. Wash. Publ. No. 101*, 3–63 (1908)
51. Mainland, G. B., *Univ. Texas Publ. 4228*, 74–112 (1942)
52. Mayr, E., *Systematics and the Origin of Species* (Columbia University Press, New York, N. Y., 334 pp., 1942)
53. Mayr, E., "The Bearing of the New Systematics on Genetical Problems: the Nature of Species," in *Advances in Genetics*, **2**, 205–37 (1948)
54. Mayr, E., *Systematic Zool.*, **3**, 86–99 (1954)
55. Mayr, E., *Trans. Roy. Entomol. Soc. (London)*, **107**, 45–66 (1955)
56. Mayr, E., Ed., *The Species Problem* (*Publ. Am. Assoc. Advance. Sci.*, **50**, 395 pp., 1957)
57. Mayr, E., Linsley, E. G., and Usinger, R. L., *Methods and Principles of Systematic Zoology* (McGraw-Hill Book Co., Inc., New York, N. Y., Toronto, Canada, and London, England, 327 pp., 1953)
58. Micks, D. W., *Ann. Entomol. Soc. Am.*, **49**, 576–81 (1956)
59. Micks, D. W., and Gibson, F. J., *Ann. Entomol. Soc. Am.* **50**, 500–5 (1957)
60. Miller, D. D., *Evolution*, **12**, 72–81 (1958)
61. Moore, J. A., *Evolution*, **3**, 1–24 (1949)
62. Müller, H. J., *Zool. Jahrb. Abt. Systematik, Ökol. u. Geograph. Tiere*, **85**, 317–430 (1957)
63. Oman, P. W., *The Nearctic Leafhoppers, a Generic Revision and Check List* (*Mem. Entomol. Soc. Wash.*, **3**, Washington, D. C., 253 pp., 1949)
64. Patterson, J. T., *Univ. Texas Publ. 5721*, 7–14 (1957)
65. Patterson, J. T., and Stone, W. S., *Evolution in the Genus Drosophila* (The Macmillan Co., New York, N. Y., 610 pp., 1952)
66. Rehn, J. A. G., and Hebard, M., *Proc. Acad. Nat. Sci. Phila.*, **67**, 292–322 (1915)
67. Richards, O. W., *Trans. Roy. Entomol. Soc. (London)*, **94**, 187–200 (1944)
68. Ross, H. H., *Evolution*, **11**, 113–29 (1957)
69. Rozeboom, L. E., and Kitzmiller, J. B., *Ann. Rev. Entomol.*, **3**, 231–48 (1958)
70. Rupert, L. R., *J. N. Y. Entomol. Soc.*, **57**, 19–48 (1949)
71. Sailer, R. I., *Bull. Entomol. Soc. Am.*, **1**, 1–34 (1955)
72. Simpson, G. G., *Evolution*, **5**, 285–98 (1951)
73. Stebbins, G. L., Jr., *Variation and Evoluiton in Plants* (Columbia University Press, New York, N. Y., 643 pp., 1950)
74. Stokes, B., *Brit. J. Animal Behaviour*, **3**, 154–57 (1955)
75. Suomalainen, E., *Hereditas*, **26**, 51–64 (1940)
76. Suomalainen, E., *Hereditas*, **33**, 425–56 (1947)
77. Thorpe, W. H., *Biol. Revs. Cambridge Phil. Soc.*, **5**, 177–212 (1930)
78. Thorpe, W. H., *Ann Applied Biol.*, **18**, 406–14 (1931)
79. Thorpe, W. H., "Ecology and the Future of Systematics," in *The New Systematics*, 341–364 (Huxley, J., Ed. Oxford University Press, London, England, 583 pp., 1940)

80. Walsh, B. D., *Proc. Entomol. Soc. Phila.*, **2**, 167–272 (1863)
81. Walsh, B. D., *Proc. Entomol. Soc. Phila.*, **3**, 403–30 (1864)
82. White, M. J. D., *Animal Cytology and Evolution*, 1st ed. (Cambridge University Press, Cambridge, England, 375 pp., 1945)
83. White, M. J. D., *Animal Cytology and Evolution*, 2nd ed. (Cambridge University Press, Cambridge, England, 454 pp., 1954)
84. White, M. J. D., *Ann. Rev. Entomol.*, **2**, 71–90 (1957)
85. Wilson, E. O., *Bull. Museum Comparative Zool. Harvard College*, **113**, 1–203 (1955)
86. Wilson, E. O., and Brown, W. L., *Systematic Zool.*, **2**, 97–111 (1953)
87. Zweig, G., and Crenshaw, J. W., *Science*, **126**, 1065–67 (1957)

ECOLOGY OF CERAMBYCIDAE[1]

By E. Gorton Linsley
University of California, Berkeley, California

Introduction

The object of this review is to bring together available information on the environmental relationships of Cerambycidae, including physiological and ethological features which may have influenced their evolution and survival. Except for larval food and nutrition, which influence host specificity, emphasis has been placed upon adult ecology, since larval habits have been summarized by Kemner (168), Craighead (77), Kojima (175), Beeson & Bhatia (21), Duffy (93, 94), and others. Although nearly 1000 sources have been consulted, it has been possible to cite less than one-third of these. In selecting items for inclusion, I have tried to emphasize the more recent or the more comprehensive of alternative references, with special consideration to those which include bibliographies. No serious attempt has been made to develop the various facets of the subject historically.

Size and Scope of Family

The family Cerambycidae comprises a group of phytophagous Coleoptera, the adults of which are commonly referred to as long-horned or longicorn beetles, the larvae as round-headed borers. Seven subfamilies are currently recognized and approximately 20,000 species have been described, although the most recent world catalogue (the parts of which are from 35 to 45 years out of date), lists only about 14,000 (Table I). Most of the genera in the larger subfamilies are small, with an average ranging from about 4 to 6.5 species per genus. One of the largest is the lamiine genus *Glenea* with nearly 600 currently recognized species in Tropical Africa, South and East Asia, and the South Pacific region (38). At the other extreme are the more than 1000 monotypic genera (Table I).

Geographical Distribution

The zoogeography of animals other than insects has been recently reviewed by Darlington (82), that of insects by Gressitt (135). The ecological aspects of the subject have been emphasized by Uvarov (314), Lindroth (200, 201), and others. Although, broadly speaking, climatic factors and availability of suitable food plants are the main factors governing the distribution of Cerambycidae in the world today [Linsley (206, 209)], past cli-

[1] The survey of literature pertaining to this review was completed in May 1958. The significant paper by W. Funk—"*Zur Biologie und Ethologie einheimischer Lamiinen*" (*Zool. Jahrb. Abt. Systematik Ökol. u. Geograph. Tiere,* **85,** 73–176, 1957)—was not available in time for consideration.

TABLE I

DISTRIBUTION OF GENERA AND SPECIES AMONG THE VARIOUS SUBFAMILIES OF THE CERAMBYCIDAE*

Subfamily	Number of Species	Number of Genera	Monotypic Genera	Per Cent Monotypic	Genera with 50+ Species	Species in Largest Genus	Average Number of Species per Genus
Lamiinae	7665	1167	503	43	22	323	6.6
Cerambycinae	4773	874	394	45	11	104	5.5
Lepturinae	799	124	62	50	1	125	6.4
Prioninae	589	109	45	41	1	53	5.4
Aseminae	54	13	8	61	0	18	4.1
Spondylinae	4	2	1	50	0	3	2.0
Parandrinae	31	2	1	50	0	30	15.5
Family	13,915	2291	1014	44	35	323	6.1

* Data compiled from Aurivillius (7) and Lameere (178)

mates, and the evolution and spread of past floras have had a profound influence on the distributional and evolutionary history of the surviving, present-day representatives of the family (214). Thus, for example, the early holarctic elements of the cerambycid fauna of the Northern Hemisphere were largely associated with the Arcto-Tertiary flora (8), which moved southward during the Tertiary period and replaced pre-existing tropical floras of the Cretaceous period. These early northern types are now represented discontinuously in Europe, Eastern Asia (Japano-Manchurian), Western North America (Vancouveran), Eastern North America (Alleghenian), and on the Mexican Plateau (206). Included are such genera as *Spondylis, Rhopalopus, Clytoleptus, Cortodera, Ergates, Rosalia, Atimia, Callimoxys, Gaurotes, Plectrura.* At least nine of these are represented in the Oligocene beds of Florissant, Colorado, by forms closely related to living species in these widely separated areas (209).

In those cerambycid groups of which the morphological relationships are sufficiently understood to permit of phylogenetic interpretation, their major patterns of distribution can readily be explained in terms of the present arrangement of the world land masses (206). The temperate fauna of the Northern Hemisphere has a large number of elements in common (Table II) which presumably were derived from relationships extending back into the Cretaceous and early Tertiary periods (133, 206, 214). At present, the distributions of these elements and of the woody plants which serve them as hosts are widely discontinuous, these discontinuities apparently reflecting segregation in the face of gradual climatic changes during the Tertiary and centers of survival during the extremes of the Cenozoic. Overlying these dis-

continuous patterns are broad Holarctic circumpolar ranges of genera and some species which apparently reflect postglacial dispersal. The vast tropical belt is extremely rich in Cerambycidae (7, 25), and although many tribes are essentially pantropical in range, this is true of relatively few genera and no species (beyond those dispersed by man) [Linsley (212)]. Thus, the tropical faunas of the Eastern and Western hemispheres appear to have been separated for long periods of time. At present they are represented in residual pockets and encroachments to the north and south of the tropics proper, especially in southeastern Asia (21) and southeastern United States (206). In North America the tropics have contributed heavily to the derived fauna of the Sonoran Desert, but apparently this has not been true of the Sahara Desert of Africa (269, 316). South temperate Ceramycidae, although having a few elements in common suggesting phylogenetic affinity, especially at the tribal level (113), generally have their own characteristics on the various continents (32, 155, 227, 260), and the faunas of intercontinental Southern Hemisphere plants appear to have been independently derived wherever they occur. Thus, the discontinuities in Southern Hemisphere distribution apparently have not resulted from contiguity of geographical origin. Several north-south, transtropical distributions are evident in both the Old and New World, but generally the Southern Hemisphere elements are so isolated morphologically as to suggest that the geographic relationship is an ancient one. Some of these may be more recent, as the similarity of certain elements in the desert faunas of western North and South America, but they need further study and analysis.

TABLE II

CONTEMPORARY GEOGRAPHICAL RANGES OF NORTH AMERICAN CERAMBYCIDAE*

	Genera		Species	
	Number	Per Cent	Number	Per Cent
World Wide	4	1.70	0	0
Holarctic	34	14.47	9	1.03
Nearctic	10	4.26	14	1.60
Alleghenian	30	12.76	264	30.28
Vancouveran	16	6.81	147	16.86
Rocky Mountain	2	0.85	39	4.47
Great Basin	0	0	27	3.09
Californian	18	7.66	45	5.16
Sonoran	44	18.72	194	22.25
Eastern Austral	10	4.26	0	0
Austro-Riparian	0	0	46	5.28
Neotropical	67	28.51	87	9.98
Totals	235	100	872	100

* After Linsley (214)

The Oriental and Papuan (New Guinea) faunas are very rich in Cerambycidae. In India alone, about 1200 species are known, more than 700 of which are Lamiinae (21), although these figures include some Palearctic elements in the Himalaya and higher mountains to the north. Gressitt (*in litt.*) has on hand an estimated 1000 species from the New Guinea–Solomon Island region, the Lamiinae far outnumbering the Cerambycinae. New Guinea has one lepturine, the Solomons none.

Australia has about 1000 known species of Cerambycidae in less than 275 genera (227). Only four subfamilies are represented, the Prioninae, Parandrinae, Cerambycinae and Lamiinae, and certain groups of Cerambycinae are lacking. Even so, Australia is also the only continent in which the Cerambycinae (550 spp.) exceed the Lamiinae (440 spp.). Prioninae are represented by 50 species in 21 genera, and flightless Lamiinae are numerous. The Aseminae and Callidiini, also predominantly northern groups, are also absent from Australia but represented in Madagascar and Chili by taxonomically isolated, presumably relict, genera. New Zealand has only about 250 known species (26, 155), the largest numbers of genera being apterous [e.g., *Somatidia, Hybolasius, Xyloteles*]. The Lepturinae, absent from Australia, are sparsely represented in New Zealand.

The Pacific islands in the area north and east of the Solomons to Easter Island and Hawaii are treated as Polynesian and oceanic by Gressitt (134). Perhaps the most interesting of the cerambycid faunas are found in the last-mentioned island group (304). Those of Micronesia are also disharmonic, lacking many of the dominant tribes in the Philippines and New Guinea and possessing largely oceanic genera with some flightless endemics (134). The Cerambycidae of the subantarctic islands, especially in the Atlantic are predominently apterous.

The longicorns of Madagascar, like other elements in the insect fauna (251), are highly endemic and diversified (e.g., the distinctive Lepturinae with many monotypic genera).

Larval Food Habits—General

Most larval Cerambycidae feed upon the solid tissues of living, dead, or dying plants or, less commonly, in rotten wood (23). For each species the limits are usually very exacting for normal development (78). Thus, the various stages of a gradually disintegrating tree have their particular species, some in the living parts, others in the recently dead material, and still others in the wood which has seasoned for several years (1, 292). As would be expected, their role in this succession varies from host to host and region to region (18, 24, 78, 83, 101, 130, 158, 166, 176, 246, 270, 281).

Many species attack living trees and shrubs, and scarcely any portion of the plant is immune. In North America, for example, smaller twigs and branches are girdled by the larvae of *Styloxus, Elaphidionoides, Aneflomorpha,* and *Xylotrechus quadrimaculatus* Haldeman (77, 78), and by the adults of *Oncideres* and *Oberea* (202, 275). Larger twigs and branches are de-

formed or killed by the larvae of *Saperda,* which cause abnormal tissue growth and gall formation (110). Some species (e.g., *Xylotrechus insignis* LeConte) bore into the living trunk through scars or the butts of broken branches. Others (e.g., *Pyrotrichus vitticollis* LeConte) destroy the heartwood of a tree but allow the living tissue to remain intact. The larvae of a number of the species which attack living trees, as *Goes, Hammoderus,* and *Saperda,* will die if the tree is cut before they mature. A few, such as *Encyclops* and *Microcyltus* (77) and *Physocnemum brevilineum* (Say) (139), feed in dry, scaly bark of living trees, causing abnormalities. Bark borers of the genus *Acanthocinus* incidentally are often very destructive to bark beetle broods (166). Species of *Prionus* may hollow out and kill the roots of living trees, *Desmocerus* those of *Sambucus* (50, 145). A comparatively few species live upon small annual or perennial plants. Thus, *Mecas* and *Crossidius* feed upon the roots of *Artemisia* and *Chrysothamnus* (211), *Tetraopes* those of *Asclepias, Anoplodera instabilis* (Haldeman) those of *Lupinus,* and *Brachysomida tumida* (LeConte) and *Cortodera berkeleyensis* G. Hopping those of *Ranunculus* (204). Finally, larvae of the prionine subgenus *Homaesthesis* feed on the roots of sod-forming grasses.

In the tropics, a greater proportion of species are live-stem borers, perhaps, as pointed out by Vogt (*in litt.*), because of the intense competition for dead wood provided by termites (and in many areas by humans). He reports dead wood cerambycids more common in mangrove swamps, where the occurrence of termites is limited.

Symbiosis and Digestion of Wood

Intracellular symbiotes of Cerambycidae have been known since the time of Heitz (144) and Ekblom (102) who observed yeastlike organisms in the cells of the intestinal walls of *Rhagium.* Schomann (283), in the most thorough study that has yet been made of their occurrence in the family, found them regularly present in the Spondylinae and Aseminae, certain Cerambycinae (Saphanini, Trichomesiini, Tillomorphini, and *Dialges,* but not other Cerambycini), and most Lepturinae, except the Toxoti, *Vesperus* and *Pidonia.* Steinhaus (299, 300) has summarized available information on the mycetomes of Cerambycidae, which most frequently consist of small tissue masses or symbiote-filled evaginations of the gut wall which circle the gut in one or two girdles, become smaller during pupation, and are lacking in the adults. However, the symbiotes are retained in intersegmental pouches of the ovipositor, smeared on the eggs during oviposition, and enter the larva as it chews the chorion during eclosion.

The possible role of intracellular symbiotes in the digestion of wood by cerambycid larvae has been a matter of controversy (47, 48, 84, 85, 219, 250), and this aspect of the subject will not be reviewed here. However, it appears that those species the larvae of which live in fresh wood of deciduous trees lack intracellular microorganisms (at least yeasts), whereas those which attack coniferous hosts, living or dead, harbor symbiotes (283).

Most of our knowledge of digestive enzymes in Cerambycidae is less than 30 years old, although an enzyme hydrolyzing xylan was found in the alimentary tract of *Phymatodes* as early as 1905. Much of the present summary is condensed from Parkin (250).

In 1930, Falck (108) and Horn (151) analysed the food and frass of *Hylotrupes bajulus* (Linnaeus) and showed that during digestion depletion of cellulose and pentosans occurs. Subsequently, Schuch (284) demonstrated that some constituent of the cell contents is necessary for the best growth of the larva, since they grow much more rapidly when feeding in the outer zone of the sapwood than in the inner zone or in heartwood. Becker (16) observed accelerated growth when larvae were inserted into wood impregnated with peptone solution, but not in wood impregnated with soluble starch or glucose. Since an intermediate value was obtained with malt extract, he suggested that a small amount of nitrogenous matter may have been present. Grösswald (136) found that, 180 days after hatching, larvae in wood impregnated with 5 per cent diastase solution weighed up to 114 mg., while similar larvae in peptone-impregnated wood weighed up to 4 mg. and in the untreated controls up to 2 mg. Parkin (250) points out that this phenomenon could hardly depend upon the enzymic action of diastase on the wood starch, since the quantity present in softwood timbers is very small, but it is possible that the diastase contains as an impurity some growth-promoting substance, the presence of which enables the larva to utilize larger amounts of some or all of the major components of the wood.

Ripper (271) has demonstrated the presence of a cellulase in mid-gut fluid of larvae of *Cerambyx, Rhagium,* and *Leptura,* and Müller (238) showed by chemical analysis that there is a considerable loss in cellulose and pentosans from wood passing through the larval gut of *Oxymirus cursor* Linnaeus, *Leptura rubra* Linnaeus, and *Gracilia minuta* Fabricius. He also detected in the alimentary canal of *Oxymirus* the enzymes cellulase, hemicellulase, xylanase, amylase, invertase, maltase, emulsin, lipase, trypsin, and erepsin. Mansour and Mansour-Bek (219, 220) have shown that the larvae of *Macrotoma palmata* Fabricius and *Stromatium fulvum* Villers possess an enzyme hydrolyzing cellulose, but that *Xystrocera globosa* Olivier cannot secrete a cellulase and appears to derive its food from the minor carbohydrate constituents which occur plentifully in the sapwood in which it lives.

Parkin (250) tested the digestive juices of six species of cerambycid larvae (Table III) and found no fundamental difference between the digestive powers of species feeding upon bark, phloem, and cambium and those living in the solid wood (although larvae of *Smodicum* seemed to be deficient in enzymes hydrolyzing the cell-content carbohydrates). Judging from his rather uniform results and those of Müller (238), it appears likely that the majority of cerambycine and lepturine larvae digest the starch, soluble sugars, hemicelluloses, cellulose, and proteins in wood. However, relatively few genera are as yet definitely known to produce a cellulase. Mansour & Mansour-Bek (219) were unable to detect this enzyme in *Xystrocera glo-*

TABLE III

THE ACTION OF THE DIGESTIVE JUICES OF CERTAIN CERAMBYCID LARVAE ON VARIOUS SUBSTRATES*

	Starch	Sucrose	Maltose	Lactose	Cellulose	Hemicellulose A	Hemicellulose B	Protein
Rhagium	++++	++++	0	+++	+	++++	++++	++++
Phymatodes	++++	++++	+++	+++	++	+++	++++	+++
Isotomus	+++	++++	+++	+++	+	++++		+++
Xylotrechus	++++	++++	0	0	+	+++	+++	+
Hylotrupes	+++	+	++	++	+	++	++	+
Smodicum	0	0	0	++	+	++++		+

* After Parkin (250)

bosa and concluded that *X. globosa* is dependent for its food on cell contents. Savely (281) tested several phloem-feeding cerambycid larvae (*Monochamus, Acanthocinus, Callidium, Rhagium, Romaleum*) for a cellulase (and for intestinal protozoa which could be considered to play a part in nutrition). The results were negative for these but positive for *Derobrachus brunneus* (Fabricius). He commented on the fact that the larvae of this species are among the largest of the cerambycids and feed in both rotten pine and oak. In pines he found them feeding on wood in a very advanced stage of decay; in oak they were feeding on the solid heartwood of a rotten log that had been dead about five years and in sapwood attacked by "white-rot" fungi. No intestinal protozoa or bacteria were found in their gut that could account for the presence of cellulase. Haliburton (139), in Canada, noted that the larva of *Physocnemum brevilineum* (Say) found partially desiccated elm phloem tissue nutritionally superior to dead and dry tissue of similar origin in outer bark. He was unable to determine what the larva assimilated from the tissue, which was negative to tests for starch.

HOST SPECIFICITY

Host specificity in varying degrees is characteristic of Cerambycidae and has undoubtedly been an important factor in their evolution [Linsley (206, 207, 214)]. Generally speaking, the more primitive groups are the more polyphagous (e.g. Parandrinae, Prioninae, Lepturinae), although some more specialized forms of restricted host range occur among them. Further, the polyphagous species are usually associated with wood which has been dead for some time or is actually decomposing. These species may be able to develop on wood of either coniferous or nonconiferous origin, but most polyphagous species are limited to either the gymnosperm or angiosperm hosts. Sometimes this distinction is made at the level of higher taxa (e.g., the Spondylinae, Aseminae, and the less specialized Callidiini are limited largely to conifers, most Lamiinae and the more specialized Cerambycinae are restricted to angiosperms). Within the Gymnospermae a sharp host-plant dis-

tinction is usually made between the group represented by the Taxodiaceae-Cupressaceae and that of the Pinaceae. Sometimes this distinction is evident at the generic level (e.g., *Atimia, Callidiellum*), and in other cases at the species level (e.g., *Callidium, Semanotus*). In general, those species which develop as larvae in living trees (e.g., certain *Saperda*) are usually the most narrowly host specific. One of the most polyphagous species is the Indian cerambycine *Stromatium barbatum* (Fabricius), which has more than 300 recorded hosts, including both broadleaf trees and conifers, but it attacks dry wood, including seasoned timber (21).

The significance of host selection among Cerambycidae and other phytophagous insects was apparently first visualized by Walsh (317), who saw in host preferences, reinforced by inheritance, the beginnings of divergence ultimately leading to separate species. His conclusions were formalized by Hopkins (150), in connection with surveys of bark beetle damage, into a principle which states, in effect, that in an insect species capable of breeding in two or more hosts, individuals will normally continue to select the particular host species in which they passed their own life cycle. If this "host-selection principle" is valid, the evolutionary results would provide, at least in the early stages, physiological strains and races with particular host preferences (306) and, subsequently, sibling species and distinct morphological species (226). However, Peyerimhoff (256) does not accept the host-selection principle, and interprets the ideas of Hopkins and Craighead as favoring the inheritance of acquired characters. Citing examples of biological races or subspecies, he concludes that host selection involves a combination of factors, including the attractiveness and condition of the host, internal factors of the plant feeder (including mutation), as well as environmental conditions. However, host selection (see below) and host specificity, although interrelated, are not necessarily the same thing. The latter defines the range within which the former usually takes place.

Experimental evidence to test these viewpoints remains largely inconclusive, in part because of the difficulty of rearing cerambycids under controlled conditions that duplicate those obtaining in the natural habitat, and in part because of problems of taxonomic interpretation. However, Crawshay (79) who found *Tetropium gabrieli* Weise exclusively on *Larix europaea* in the wild state, when he confined females with *Picea* and *Pinus*, was able to force successful attack and obtain subsequent larval development. Craighead (76) designed a more comprehensive series of experiments in order to test the application of the basic host-selection principle of Walsh and Hopkins in the family Cerambycidae and came to the following conclusions:

(1) In practically all the species experimented with, the adults show a marked predilection for the host in which they have fed as larvae, provided they are not deterred by other factors, such as the unfavorable condition or the small quantity of the host; (2) there is considerable variation in the degree of preference for the original host as between different species, thus—(a) certain species are capable of

living in only one genus or species of plant, which consequently they select; (b) certain species, chiefly those living in nature in several hosts, can be forced to adopt a new host; (c) certain species, chiefly those feeding in nature in a great variety of plants, show little discrimination in the selection of hosts; (d) certain species feeding in nature in a great variety of hosts often show a preference for a few of these; (3) in forced transference of individual adults of a species to a new host, a high mortality of the broods usually occurs, especially in the case of eggs laid by beetles emerging from the original host, in which case the mortality is often total; one-half to full-grown larvae, however, usually can be successfully transferred to a new host and live and transform to adults; (4) with some species that can be reared in a secondary (new) host, by the larvae feeding one or part of one year, preference for that host is shown by the resulting adults; (5) in general, the fewer the hosts in nature, the more marked the predilection for a particular host, and vice versa; (6) continued breeding in a given host intensifies the preference for that host; (7) the condition of the host has a great influence on host selection, in that every species prefers an optimum condition of the host which it selects and will choose a new host in the optimum condition in preference to an old host in which the conditions are unfavorable; (8) the quantity of wood at the disposal of the ovipositing adults may influence the insects in their choice between different kinds of host wood, in that, if there are many adults to a limited amount of the primary host, some species will select a secondary host if such is available; if this is done, however, the resulting brood is weakened.

Duffy (93), utilizing the results of rearing experiments and field observations on British species, came to somewhat different conclusions:

(1) Ovipositing adults are influenced to a greater degree by the nature and condition of the host (i.e. thickness of bark, stage of decay, moisture content) than by the identity of the host species . . . ; (2) ovipositing adults (at least under laboratory conditions) are influenced by the amount and variety of hosts, irrespective of species . . . ; (3) the successful development of larvae depends to a far greater extent upon the nature and condition of the wood than upon the host species concerned . . . ; (4) many species [show] a marked preference for a particular part of a tree . . . and (5) the range of hosts is, in general, greatest in species which oviposit on freshly cut, slightly injured or decaying wood; whereas the hosts of species which oviposit only on healthy living wood or plant tissue are considerably restricted, often to a single genus.

Much more data, derived from both field and laboratory experimentation, are needed on the subject, especially in relation to species associated with living and freshly cut trees. Special attention should be given to sibling species, host strains, and geographical races in such groups as *Callidium, Xylotrechus, Neoclytus,* and *Saperda.* There is reason to believe that certain "species" which appear to have a wide host range may actually consist of a series of sibling species or potential sibling species in the form of host strains. There is much evidence in the Cerambycidae of abrupt and sudden changes of host plants (e.g., the species of *Atimia* are limited to the Taxodiaceae and Cupressaceae, but the closely related *Paratimia conicola* Fisher lives in pine cones). Further, closely related sibling species are usually sympatric with quite different host plants, but closely related

allopatric species usually have identical or closely related host plants. This suggests that host specificity may sometimes result in sympatric speciation.

Generally speaking, the host-specific Cerambycidae are sympatric with their host plants, but the two ranges rarely coincide. Discussing the distribution of forest Cerambycidae in India, Stebbing (298) points out that the habitat of the same species of tree may vary from a comparatively hot, dry climate to a hot, moist one, as the sal (*Shorea robusta*) in the Central Provinces and in Assam. Some of the species which infest the tree in the hot, dry climate have also become adapted to the hot, moist one. Although *Hoplocerambyx spinicornis* (Newman) infests this tree both in the Central Provinces and in Assam, it extends southward far beyond the range of the sal to attack *Duabanga sonnatioides* and *Pentacme suavis*. On the other hand, the range of *Hoplocerambyx* does not extend with the sal tree into Northern India; in the foothill forests of the Western Himalaya, it is replaced by the related longicorn *Aeolesthes holosericea* (Fabricius).

The evolutionary significance of host specificity in common with partial geographic isolation is evident in the Hawaiian Islands. Nearly 75 species of *Plagithmysus* and a few other very closely related genera are now known in this island group, the whole complex presumably having been evolved from a single clytine ancestor, possibly from America (323). The species are highly host specific, attacking dead and dying branches or sickly and fallen trees, and each is confined to a single island (303, 304). Thus, for example, the common and widespread *Acacia koa* is attacked by at least six species of *Plagithmysus* (two each on Oahu and Kauai, one each on Maui and Hawaii) and 11 species of *Neoclytarlus* (three each on Kauai, Maui, and Hawaii, two on Oahu). A similar relationship exists between *Pipturus pelea,* and other endemic Hawaiian trees, and various species of *Plagithmysus, Callithmysus, Paraclytarlus,* and *Neithmysus* (the last restricted to *Pelea*). By contrast, most of the Cerambycidae which have been introduced to the islands through commerce are associated with introduced lowland trees, and the two endemic stocks of more generalized, polyphagous Cerambycidae, *Parandra* and *Megopis,* have but a single species each throughout the islands.

Economic Importance

The Cerambycidae comprise one of the economically most important groups of insects of the world (4). Since their economic importance is a reflection of their ecology, a brief review of the ways in which they compete with man is here included [for more detailed accounts see Duffy (93) and Linsley (212)].

Forests.—Cerambycidae, in the larval stage, are primarily borers in dead wood, and their ecological role, at least in the temperate forest, involves the reduction of dead and dying trees, broken branchs, and slash. Likewise, a great number of neotropical species have "pruning" habits, and contribute to the "littering" of the forest. In the economy of the forest, these are beneficial roles. But when storms or fires sweep through forested regions

and blow down or scorch commercial timber, Cerambycidae may destroy the wood before it can be utilized (78) or may develop in salvaged lumber (169) and subsequently emerge from structural timber (100). Among species which attack living trees, many cause serious injury or death or attack wood with a high economic value to man (78, 106, 166, 293). Thus, one small outbreak of one species, affecting only eight square miles of forest, killed 45,000 trees aggregating nearly 1 million cubic feet of timber (4, 212). Cerambycids have also been implicated in the transmission of diseases of forest trees. Reid (267) records the association of *Monochamus* feeding scars with die-back of balsam fir. Isolations from the twigs involved yielded three fungi, *Thyronectia balsamea* (Cooke & Peck), *Micropera abietum* (Peck), and *Cystospora* sp.

In tropical regions, forest species are less well known, except in India, Burma, and Ceylon, where they are regarded as the most destructive group of forest insects. Beeson & Bhatia (21) have summarized data on 350 species associated with 568 species of trees, shrubs, and woody climbers. The sal tree has 37 species which attack it, and one dry wood borer, *Stromatium barbatum* (Fabricius), has 311 known food plants. More than 100 species injurious to forest trees in South America are enumerated by Costa Lima (70) and Bosc (32); numerous African species are recorded by Duffy (94).

Forest products.—Cerambycid larvae may cause serious defects in lumber, either by attacking living trees or by attacking shortly before cut logs are sawed. In temperate forests, the resultant reduction in grade probably causes a greater monetary loss than do the tree-killing species (78). Further, defects in living trees are frequently enlarged by other insects until the heartwood is completely destroyed.

Damage to seasoned lumber by round-headed borers may occur where lumber is seasoned or stored in open yards near forests (231). But since very few species oviposit on dry, barkless wood, few become domestic pests. An exception is the "old house borer" or "Hausbock," *Hylotrupes bajulus* (Linnaeus), which feeds in the sapwood and to a lesser extent in the heartwood of dry, seasoned, coniferous timber such as telephone poles, fences, and roof and attic supports (93, 97). The Indian *Stromatium barbatum* (Fabricius) also attacks wooden structures, including furniture, supporting timbers, panels, shelves, etc. (21, 105), as does the East Asiatic *Stromatium longicorne* Newman, especially in Taiwan and the Ryukyu Islands [Gressitt (*in litt.*)]. A number of other cerambycids infest wood prior to utilization and cause damage through subsequent emergence (77, 93, 96, 100, 153, 189, 205). Structural timbers in contact with the ground are also subject to direct attack, especially when old and moist (216, 218, 247).

Shade trees.—In temperate regions, round-headed borers usually attack shade trees only when other factors, such as defoliation, diseases, drought, frosts, or transplanting make them susceptible to attack (109, 146), and it is often difficult to place responsibility for the death of the tree (78).

However, Pechuman (252) considers feeding by adults of *Saperda tridentata* Olivier of primary importance in the transmission of Dutch Elm disease, and *Anoplodera nitens* (Förster) has been charged with carrying the spores of chestnut blight (131). In tropical regions, living shade and ornamental trees are attacked by a large number of Cerambycidae (21, 70, 94).

Fruit and nut trees.—Trees grown for fruit and nut crops, both native and introduced, are also widely attacked by cerambycids. Of 118 kinds of injurious Cerambycidae in Japan, 49 are associated with fruit and nut trees, including mulberry, and four with grape vines (293). In Australia (185), parts of Asia (86, 159), and in North America (77), the more important pome fruits are attacked by species formerly associated with native trees, as the *Amelanchier*- and *Crataegus*-infesting apple-tree borers (*Saperda* spp.) of New England (40, 147). In most areas where grapes are grown, various endemic Cerambycidae attack the vine (77, 225, 293). Several species of *Oberea* girdle stems of fruit and nut crops, one of the best known being the raspberry cane borer, *Oberea bimaculata* (Olivier), which is also injurious to perennial asters (156). Although fruit-tree injury is caused by larvae, adult Cerambycidae may scar the fruit surface (170) or burrow into soft fruits, such as ripening peaches (137).

By far the most important cerambycid pests are those which attack living tropical and subtropical fruit and nut trees (132, 163, 230, 241, 265, 266, 289), including cloves (162), coffee (68, 70, 196, 223, 315), cacao (68, 70, 272), fig (138, 152, 157, 164, 293) and citrus (57, 261, 288, 305, 318). Forty-eight species attack native and introduced species of *Coffea* in tropical Africa, and native species have taken to *Citrus* in most of the areas where it has been introduced. In eastern Asia, at least four of these are major pests (65, 132, 148, 198, 199), one of which, *Anoplophora macularia* Thomson, kills thousands of young trees [Gressitt (*in litt.*)] and in some areas infests 90 per cent of the remainder. Many other species are polyphagous and have turned to citrus from distantly related hosts (10, 70, 72, 95, 154), but in Australia *Citriphaga mixta* Lea overlaps from the desert kumquat (119) and *Uracanthus cryptophagus* Olivier from the native finger lime (244). Of the several species of longicorns attacking citrus in tropical Asia (66), some of the Callichromini are probably native to that host (177, 239, 263).

Although several polyphagous Cerambycidae attack palms in various parts of the world, relatively few species are regularly associated with this group of plants (190). At least three attack coconut in the South Pacific, and in Micronesia larvae of *Caroliniella aenescens* Blair bore in the petioles and midribs of the fronds while the adults feed in the unfolding buds (134). A few longicorns are associated also with the date palm in Asia Minor or North Africa.

Vegetable and field crops.—As pests of vegetable and field crops, cerambycids are less well known. Species of *Phytoecia*, which live in the stems of umbellifers and composites are capable of severe injury to seed carrots

(93, 167) and to Jerusalem artichoke (293), and have been accused of damaging cabbages (131). Other Lamiinae infest stems of sweet potato in Java (164), those of eggplant in British Guiana (67), and those of cucurbits in Argentina (32). *Ropica dorsalis* Schwarzer and three species of *Apomecyna* bore in watermelon, muskmelon, and cucumber vines in China or Japan (132, 293, 294), and related species attack stems of French beans in Portuguese East Africa (280), of pumpkins and melons in the Sudan (259), South Africa (121), and Queensland (224). In southwestern United States, the larvae of *Dorcasta cinerea* (Horn) bore in stems of sunflower and other oil-seed crops, as do those of *Agapanthia dahli* Richter in the Ukraine (248). Similarly, *Ataxia hubbardi* Fisher infests stalks of cotton in Texas (234), as does *Tragiscoschema bertolonii* Thomson in Mozambique (197), and *Tragiscoschema wahlbergi* Fahr and *Volumnia westermanni* Thomson in Portuguese East Africa (280). In southern Europe, stems of wheat, barley, and rye are fed upon by larvae of *Calamobius filum* Rossi (131), and in North America the related *Hippopsis lemniscata* (Fabricius) attacks the stems of *Vernonia* and other plants (286). Stems of jack beans in Hawaii are infested with *Sybra alternans* Wiedemann (301).

A number of subterranean forms are injurious to turf and grass plots in North America (77) and Europe (131). The roots of maize are damaged by *Dorcadion* in Italy (131), those of sugar cane in China by larvae of *Philus* (132) and elsewhere by various Prioninae and Lamiinae (34). In the Mediterranean region, larvae of *Vesperus* are especially injurious to roots of alfalfa, root vegetables, and potato tubers (131). Other larvae feed in the roots of *Ferula* in Afganistan [Vogt (*in litt.*)] and sever the roots of hemp in Japan (175). Dry derris roots are also attacked by numerous Cerambycidae both in Asia (232) and in South America (90).

Seed-infesting species.—Although seeds of various plants, in particular conifers and legumes, are attacked by a great variety of insects, few are regularly utilized by Cerambycidae. A North American and an Indian species infest full-grown green or maturing pine cones (54, 111). *Lophopoeum timbouvae* Lameere feeds in the seed pods of various legumes in South America (32, 43, 71), as does a species of *Leptostylus* in Puerto Rico (322). A related species lives in the fruits of *Sapindus* in Brazil [F. Lane (*in litt.*)], and *Baryssinus leguminicola* Linell in the seeds of *Enterolobium* in Paraguay (64). In North America, *Lepturges spermophagus* Fisher infests unripened seeds in green pods of cowpeas in Mexico (112), and *Ataxia sulcata* Fallén and *Leptostylus terraecolor* Horn attack mangrove in Florida (77). In Africa, several small lamiines develop in dry coffee berries (3, 191), along with *Enaretta castelnaui* Thomson (94).

Orchids and flowers.—In Java, a larval *Diaxenes* lives in the fleshy roots of various orchids and the adults feed on young leaves, flowers, and fruits (117). Related species infest orchids in Burma and the Philippines (122, 302). Garden plants, especially those with woody stems, as roses and crotons, are also subject to attack (142, 287), as are asphodels, hellebores, and

euphorbias (131, 316), and also ornamental umbellifers, borages, and composites (131, 132, 293).

Opuntia cactus.—Cerambycidae have been intentionally introduced into Australia and South Africa to combat escaped cactus in range lands (88, 254, 255). Two species of *Moneilema* and a *Lagocheirus* were introduced into Australia from Texas and Mexico and apparently established, but proved of minor importance (88). *Moneilema ulkei* Horn was reared in South Africa but apparently never released (191). *Lagocheirus* were released, but the populations have continued to decline. Weber (319), Dodd (89), and Fullaway (120) record the release of *Moneilema* and *Lagocheirus* from Australia on the island of Hawaii to suppress the tree cactus, with indications that *Lagocheirus* at least, would become established, as it has in Australia (192). More recently, *Aerenicopsis championi* Bates and *Plagiohammus spinipennis* (Thomson) have been introduced from Mexico as potential agents for the biological control of lantana [Weber (319); Gressitt (*in litt.*)].

Ethological and Physiological Adaptations of Adults

Emergence and seasonal distribution.—In normal years, emergence is characterized by a definite succession of forms. For instance, in California, at lower elevations and in the more southerly parts of the state, the first species appear in February and March (occasionally earlier) and include *Neoclytus conjunctus* LeConte, *Cortodera* spp., *Atimia* spp., and *Lophogogonius crinitus* (LeConte). These are followed in late spring and early summer by species of *Brachysomida, Anoplodera, Leptura, Xylotrechus, Synaphaeta,* and others. In midsummer only a few new forms—including *Methia, Styloxus,* and *Aneflomorpha*—first appear, but in the fall there is a renewal of activity with the emergence of *Crossidius* and the second broods of *Atimia dorsalis* LeConte and *Saperda hornii* Joutel, etc. At higher elevations and in the northern and cooler parts of the state, the season is much shorter. The earliest forms, such as *Rhagium* and *Spondylis,* usually do not appear until May or June, and the late forms begin to disappear in August. In eastern North America the majority of species are active in June and July (249). However, the fall-emerging *Megacyllene robiniae* (Forster) may be found as late as November (173). In India, four emergence periods, based on climatic factors, have been defined by Beeson & Bhatia (21). The temperate summer-emergence period involves various endemic genera found in the mountains which attack living trees, as well as dead wood borers in genera with northern, Holarctic affinities [e.g., *Arhopalus, Purpuricenus, Tetropium, Molorchus, Prionus*]. The pre-monsoon or dry-season emergence period of the submontaine and plains regions brings forth such species as *Glenea spilota* Thomson (Fig. 1); the monsoon period, initiated by the southwest monsoon, and the post-monsoon season or autumn of South India each are characterized by the emergence of certain species. Those that emerge at the beginning of the monsoon period are influenced by the date of arrival of the monsoon and the quantity and distribution of the rainfall.

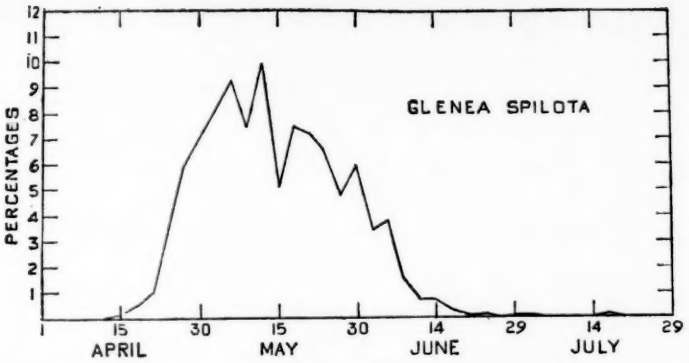


FIG. 1. Emergence of *Glenea spilota* Thomson, a dry season species, plotted at three-day intervals. [After Beeson & Bhatia (21)] Compare with *Hoplocerambyx spinicornis* (Newman) (Fig. 2, 3), a typical wet-season species.

Thus, 10 inches of rain will bring forth from 97 to 100 per cent of the adults of *Stromatium barbatum* (Fabricius) and from 30 to 59 per cent of *Hoplocerambyx spinicornis* (Newman). When 30 inches of rain have fallen, 93 per cent of the *Hoplocerambyx* will have emerged (Fig. 2). However, since monsoon precipitation is uneven, emergence is uneven, and the beetles respond to each shower or storm (Fig. 3).

Longevity.—Little is known of the natural longevity of emerged adult longicorns. The limited feeding of many species suggests that they are probably short-lived. Craighead (73) reports that certain Prioninae have been kept alive without food for 30 to 40 days, but so long a life span is probably exceptional for the family. Beeson & Bhatia (21) were able to keep adults of *Hoplocerambyx spinicornis* (Newman) alive for 10 to 14 days without solid food or water. Still, water is necessary, and without it adult life is reduced by 10 days or more. The normal adult food is bark, particularly living inner layers, and sap, which is imbibed until the alimentary canal is fully distended. After gorging on sap the beetles appear intoxicated and often are unable to stand or fly. The longest life of adults in captivity was 49 days for the male, 38 days for the female (Fig. 4). Under laboratory conditions, with food provided, the writer has found various Aseminae and Cerambycinae relatively short-lived, often surviving only two or three weeks, in which time mating and oviposition took place. Some of the Lamiinae, however, survived for several weeks under laboratory conditions, and there are indications that adults of this subfamily are generally longer lived. With food provided, adults of *Diaxenes* have survived for three months in the laboratory (117), and under optimal conditions those of *Dihammus cervinus* Hope may live for 17 weeks and *Batocera rufomaculata* DeGeer for eight months (21). In the Cerambycinae, males are often

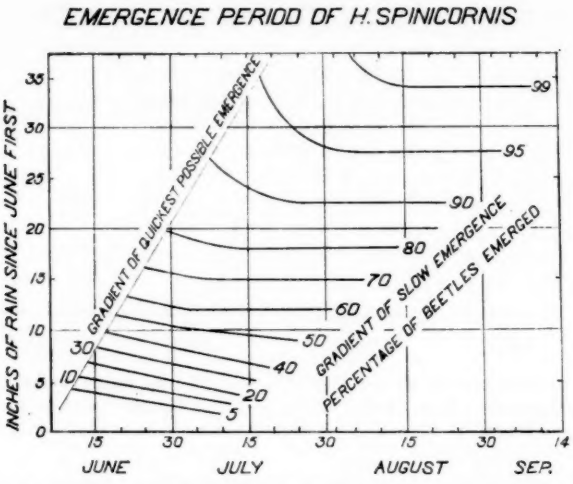


FIG. 2. Correlation between number of inches of rain falling since June 1, the date, and the percentage of the total annual population of *Hoplocerambyx spinicornis* (Newman) emerged. [After Beeson & Bhatia (21)]

shorter lived than the females, and after they have accomplished their sole function, that of fertilization, death may ensue rather quickly. However, those of *Hylotrupes bajulus* (Linnaeus) live on the average about twice as long as females (e.g., 16:8 days). At low temperatures (11°C.) the life of both sexes is prolonged and the difference less marked (97).

Longevity in adult Cerambycidae can also be profoundly influenced by

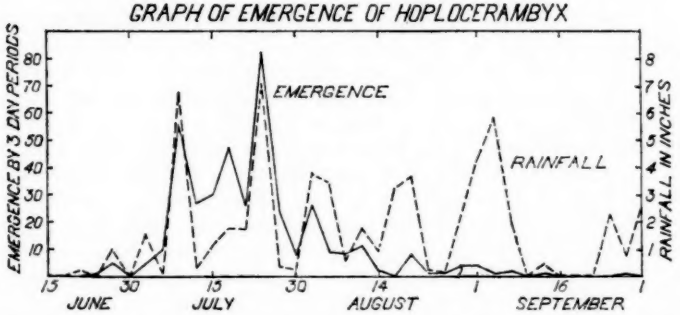


FIG. 3. Correlation between emergence of adults of *Hoplocerambyx spinicornis* (Newman) at three-day intervals and rainfall for the same intervals. [After Beeson & Bhatia (21)]

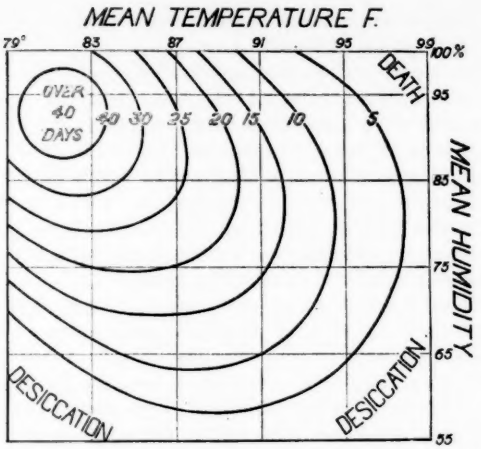


FIG. 4. Zones of maximum life of adult beetles of *Hoplocerambyx spinicornis* (Newman) at different combinations of temperature and relative humidity. [After Beeson & Bhatia (21)]

physical factors in the environment. Thus, *Hoplocerambyx spinicornis* (Newman) finds low humidities unfavorable at both low and high temperatures, and in dry air the beetle dies of desiccation (Fig. 4). High temperatures speed metabolism and reduce life proportionately. For this species, the most favorable temperatures are in the vicinity of 80°F. at relative humidities of 88 to 98 per cent.

Feeding.—Although some Cerambycidae (e.g., *Hylotrupes, Stromatium*) appear to require little or no food in the adult stage, for most species some feeding appears to be an essential prerequisite to egg maturation and oviposition. According to Schwarz (285), *Dendrobias* are especially fond of printer's ink and sometimes obliterate the large letters on advertising posters. *Monochamus* have been accused of injury to rayon dresses (128). Most species, however, utilize more conventional foods, and have been classified by Butovitsch (51) as follows: (*a*) flower feeders on pollen, stamens, nectar, etc. [most Lepturinae, certain Cerambycinae, a few Lamiinae]; (*b*) bark feeders on thin bark, bast, twigs, branches, trunks [Lamiinae]; (*c*) leaf feeders on foliage, leaf stalks, etc. [Lamiinae]; (*d*) pine needle and cone feeders [e.g., *Monochamus, Acanthocinus*]; (*e*) sap feeders [some Lepturinae (e.g., *Rhagium*), many Cerambycinae]; (*f*) fruit feeders [a few Cerambycinae, many Lamiinae]; (*g*) root feeders [*Dorcadion, Vesperus,* etc.]; and (*h*) fungus feeders [certain Lepturinae and Lamiinae].

Flower feeders are especially attracted to Umbelliferae, Compositae, and Rhamnaceae (143), although the Javanese lamiine *Prosoplus banki* (Fabri-

cius) is attracted to male flowers of *Zea mays* (164). The floroecologist Lovell (215) regards the lepturine Cerambycidae as perhaps the most significant group of anthophilous Coleoptera. But whereas his predecessors, including Müller (237) and Knuth (174), regarded the narrow body form, the elongated, posteriorly constricted head, the anteriorly narrowed pronotum, and the hairy maxillae as adaptations developed to assist in the extraction of nectar and pollen, Lovell, while granting the usefulness of the structural modifications, regards them as nonadaptive. In fact, he claims that the genera of Lepturini were probably essentially differentiated before they became anthophilous, a conclusion that is difficult to accord with known facts regarding the age and differentiation of the group.

Flight.—Since by far the majority of cerambycids fly at dusk, or during the night, or in the early morning, especially in the warmer and more tropical regions, flight behavior in this family has yet to receive systematic study. In more northern regions [e.g., Scandinavia and the British Isles], most of the species fly in the bright sunshine (93, 168). Some of the flower-visiting forms are regarded as the most skillful fliers, and in general the more brightly colored, diurnally active Cerambycinae are faster of movement than the nocturnal forms. Nonetheless, the flight of by far the majority of the members of the family is relatively slow, quiet, and more or less direct, although many of the larger types, especially in the Prioninae, are lumbering, awkward and noisy fliers.

Assembling and mating.—Males of a given species usually emerge before the females and are the active agents in sex location. In many Cerambycinae, copulation may take place shortly after emergence from the pupal cell (28, 321) but in the Lamiinae usually awaits an interval of adult feeding (21, 264). Since the site of mating, except in flower-visiting types, is usually on the host plant, sex location and host selection are sometimes intimately associated, and the sex location may precede and determine the choice of host. Sex location is aided by olfactory organs in the antennae, and males may be attracted over long distances to tethered females (258). But while the olfactory sense appears to guide the males to the immediate vicinity of the females, other senses may play a role in the final contact. Heintze (143) found that males of flower-visiting Lepturini recognized females only by touching them with the antennae and lost interest in them after both antennae (but not one or simply the outer portions) were removed.

Apparently all members of the cerambycine tribe Callichromini produce a penetrating sex attractant variously described as resembling musk or attar of roses. The aromatic glands are located on the metasternum near the hind coxae (282, 295) and are present in several of the tribes of the cerambycine series (186). The scent emitted by the willow-feeding *Aromia moschata* (Linnaeus) is said to contain salicylic ester (149) and is not only detectable by humans at a distance of several yards (92) but is apparently capable of "stupifying" certain Diptera (104). *Mallambyx raddei* Blessig also appears to be odoriferous [Gressitt (*in litt.*)]. The lamiine *Agapanthia villosoviri-*

descens (DeGeer) emits a powerful scent which is said to resemble the smell of "snuffed candles," whereas the South African cerambycine *Xystrocera nyassae* Gahan possesses a fetid stench (93). On the other hand, Kalshoven (164) compares the scent emitted by Indonesian *Xystrocera* spp. with the musky odor of *Pachyteria* and *Chloridolum* (Callichromini). The Australian species of *Syllitus* emit a strong smell of carbolic when disturbed or captured, but this may be a defensive action unassociated with sex attraction (227).

The act of copulation takes place at night or late in the day in most Prioninae, Aseminae, and more primitive Cerambycinae, and during bright sunlight among the Lepturinae, Clytini, and Callidiini [cf. Rungs (277)], although there are many exceptions [cf. Cheron (60)]. Copulation behavior has been described by Beeson & Bhatia (21), Butovitsch (51), and others. After copulation and during oviposition, males of many species maintain attendance upon the females in a position of amplexus, suggesting that repeated matings take place. Among the Clytini the same pairs have been observed to copulate several times in a period of 10 to 15 minutes (51, 98). Since cerambycids cannot be disturbed readily during copulation and subsequent attendance, these facts assume considerable ecological importance.

Competition for females is often violent among the Prioninae and Cerambycinae, and mutilation is particularly common among males of these and other longicorns with prognathous or partially prognathous mandibles (21, 98, 233). The Lamiinae, which have vertical or retracted mandibles, usually butt or push one another until one of the opponents departs (240, 320). In *Hylotrupes,* the sexes attack each other before mating, and records exist of "courtships" in which all the legs of a female were amputated (97). In captivity, large males of *Hoplocerambyx* have been observed to monopolize several females, driving off smaller males (21).

In *Monochamus,* males of one species have been reported in attendance upon females of another (235), and two species may sometimes behave as one population, occurring on the same host at the same time and place (125). Interspecies matings and attempted matings have also been reported in the Lepturini (258), as has an interfamilial mating between a male cantharid (*Rhagionycha fulva* Scopoli) and a female cerambycid (*Clytanthus herbsti* Brahm) [Niesiolowski (243)].

Males of certain Cerambycidae, especially Clytini are attracted in late afternoon and evening to the leaves of certain broadleaved plants, such as *Verbascum,* which do not serve as host plants and are rarely visited by the female (297). Beeson & Bhatia (21) found 96 species in 58 genera consistently frequenting the foliage of sandal (*Santalum album*). These were diurnal, sun-loving species which do not feed on sandal as larvae or adults.

Attraction to host plant.—The facility with which host selection is accomplished in this family is undoubtedly a result of high development of special sense organs. Most of these organs are found in the antennae. Although the loss of a single antenna appears to have little effect on either

sex location or host selection, the loss of both structures greatly impairs or inhibits both of these functions. Without any doubt, the olfactory sense is one of the most important concerned. There is a definite succession of species which visit timber after cutting, and the species which arrive a day or so after felling are quite different from those which appear a week or two later. Apparently the chemical changes which take place in the wood have a selective influence upon the beetles. Bait pans designed for the attraction of Cerambycidae likewise exhibit selectivity in different stages of fermentation.

The olfactory sense is well developed in the Cerambycidae and several authors have commented on the speed of their reaction to certain odors. In particular, Stebbing (298), Beeson (19), and Beeson & Bhatia (21) have emphasized the immediate response of *Hoplocerambyx spinicornis* (Newman) to the fresh sap of *Shorea robusta,* to which adults fly from considerable distances; they have been attracted over a measured distance of a quarter of a mile within five minutes, flying upwind. Single trap trees have attracted 836 adults over a period of several days, 465 in a single day. The beetles approach upwind, the males preceding the females. Since such trees attract for only a quarter of a mile or so to the leeward and are almost nonfunctional to the windward, the numbers indicate considerable flight activity by the beetles while seeking an appropriate host.

Fermenting baits have long been used as a means of attracting adult Cerambycidae (55, 56), as have natural sites of oozing sap (311) or blazes especially prepared (307). Chemsak (59) records the attraction of large numbers of both sexes of *Dendrobias mandibularis* Serville and two females of *Eustromula validum* (LeConte) to watermelon rinds at a roadside picnic stand in Arizona.

Fermenting baits are most attractive to species which normally infest broadleaved trees and shrubs, less so to species which breed in conifers. However, Becker (16, 17) found pine oils containing the terpines α-pinene and β-pinene attractive to ovipositing females of *Hylotrupes,* and Gardiner (126) succeeded in attracting large numbers of conifer-infesting species in Quebec with turpentine.

Infestations of *Saperda calcarata* Say in poplar are often characterized by "brood trees" (253). Such trees are usually larger than average for the stand and have shown good growth. Once such trees become infested, the borer population builds up rapidly and heavy re-infestation occurs until the host is no longer suitable for larval development. Dead trees are never attacked by this species, and only late-stage larvae can survive in cut wood.

Sound production.—Most adult Cerambycidae possess the power of stridulation (222). In the Aseminae, Cerambycinae, Lepturinae, and Lamiinae, this sound is produced by friction between the inner edge of the posterior margin of the prothorax and a specialized striated area on a median anterior prolongation of the mesonotum (91). This stridulatory plate varies from group to group and has been extensively used in the classification of genera and tribes (186), less commonly at the species level (207). In the huge

North African lamiine *Petrognatha gigas* (Fabricius), which produces a "vigorous, but otherwise harmonious stridulation," the area bears some sixty transverse striae that are very close together; in *Phryneta aurocincta* Guérin, which produces an extremely shrill sound, the plate is very elongate and from 180 to 200, very fine, close, distinctly elevated striae are involved (191). In the subfamily Prioninae, no mesonotal stridulatory plate is present, and sound is produced by rubbing the posterior femora against the edge of the elytra (221). The Hawaiian clytine genus *Plagithmysus* utilizes both methods of sound production (290), and a special "drum" has been described in *Nothorhina* by Faber (107).

Since both sexes usually stridulate when captured, the function is commonly regarded as defensive [Duffy (93)], but it is possibly involved in sex location also [Alexander (2)]. Beeson & Bhatia (21) state that males of *Hoplocerambyx spinicornis* (Newman), when courting, sometimes raise the body to the full extent of the legs and stridulate. In the Madagascan genera *Ranova, Leucographis,* and *Lasioceris,* the striae are coarser in the male (123). Sexual dimorphism in the stridulatory apparatus is also evident in some of the Lepturini [Kaszab (165)].

Cheesman (58) records that vast numbers of longicorns were attracted to a girdled tree in the Society Islands and in flight produced a humming so loud that they were mistaken for a swarm of bees. Similar observations have been made elsewhere, suggesting that the sound may assist in host attraction.

Oviposition.—Butovitsch (51) has made a careful analysis of the oviposition habits of the Cerambycidae and has proposed two major types. The first is: *oviposition exclusively with the aid of the ovipositor* (most Parandrinae, Prioninae, Spondylinae, Aseminae, Lepturinae, Cerambycinae), either (*a*) on the bark or outer surface of the host [e.g., *Spondylis* and a few Cerambycinae] or (*b*) in bark cracks or under bark scales [usual method for most forms which utilize the ovipositor only] or (*c*) in cracks and crevices in wood [a few Aseminae and Cerambycinae, e.g., *Arhopalus, Hylotrupes, Stromatium*] or (*d*) in entrance, emergence or ventilation holes of other insects [e.g., certain Prioninae, Lepturinae, Cerambycini, etc.] or (*e*) in the soil [primarily Prioninae] or (*f*) on the surface of various objects other than the host [*Vesperus*]. The second type is: *oviposition with the aid of the ovipositor and mandibles* [Lamiinae], either (*a*) in egg niches without special preparation of the substrate [most Lamiinae]—in which case they may be (*1*) in egg niches prepared by the mandibles [e.g., *Plectrodera, Phryneta, Oncideres, Saperda,* etc.] or (*2*) in egg niches prepared by the ovipositor and mandibles [*Acanthocinus*]—or (*b*) in egg niches cut by the mandibles or drilled with the ovipositor with special preparation of the substrate [certain *Phytoecia, Oberea,* gall-forming species of *Saperda*].

Thus, the least specialized groups morphologically have the simplest oviposition habits; in some of the Prioninae and Lepturinae little more than imbedding the eggs in soft, decomposing wood is involved. The most spe-

cialized habits are found in the Lamiinae, which frequently utilize the mandibles to prepare the oviposition site. Trägårdh (313) associates this habit with the form of the lamiine head, which, with the retracted face and unusually powerful, somewhat oblique mandibles, is one of the basic features of the subfamily.

Although each of these methods of oviposition has special ecological significance, perhaps the most interesting is the girdling habit of the *Oncideres* and their relatives. The parent beetles completely girdle twigs and branches (some species use only those an inch or two in diameter) oviposit in the section beyond the girdle, thus providing freshly killed tissue in which the larvae can develop. Certain species, as *Oncideres dejeanii* Thomson, transversely groove or scar the bark adjacent to the egg site to loosen it for the young larva (180). In temperate regions the twigs and branches usually remain attached to the trees during the summer months and obtain moisture from the tree; they fall to the ground in the autumn winds, when they may be protected during the winter by a covering of leaves. Linsley (208) has pointed out that this habit has considerable ecological significance for other wood-boring insects which avail themselves of the niche provided by *Oncideres*. Fonseca (115), Bruch (44), Bosc (32), Lane (180), and Bondar (30) have briefly discussed the habits of *Oncideres* ("serradores") which girdle Argentine and Brazilian fruit and forest trees. Although some species have wide host ranges, many are associated with native Leguminosae. The North American species also (208) are largely attached to legumes, but one species attacks oaks, and the notorious "twig girdler," *Oncideres cingulatus* (Say), is a serious pest of pecan, hickory, and persimmon, and certain other fruit and ornamental trees. *Oberea, Nupserha,* and relatives are also twig girdlers and include numerous species of economic importance in Europe (9, 245) and Asia (99).

Overwintering and hibernation.—Overwintering among the Cerambycidae of temperate regions is normally accomplished by the immature stages or by unemerged adults within the pupal cell. Although Duffy (93) claims that "strictly speaking, cerambycids do not hibernate," *Apomecyna binubila* Pascoe in South Africa (121), *Aulaconotus pachypezoides* Thomson in Japan (175), and possibly the European *Pogonocherus hispidus* Muller (46), as well as several North American species [mostly wingless Lamiinae of the genera *Ipochus, Plectrura, Moneilema* and *Psenocerus* (140, 187, 204)], are known to live as adults from one season to another. Adults of other Nearctic species are occasionally encountered in the winter months, particularly in the warmer areas, but these usually may be accounted for by abnormally early emergence (204). Even in British Columbia, *Atimia dorsalis* LeConte has been taken in flight as early as March 20 (188). Winter activity of adult cerambycids is common in southern Florida (27) and other areas where freezing temperatures seldom occur.

Structural Adaptations of Adults

Body size and form.—Cerambycids include some of the smallest and the largest beetles known. In North America, *Cyrtinus pygmaeus* Haldeman may be less than 2 mm. long, and *Prionus californicus* Motschulsky and *Ergates spiculatus* LeConte occasionally exceed 60 mm. The largest European species, *Cerambyx cerdo* Linneaus, attains more than 50 mm. But it is in the tropics that species attain the greatest size. The South American *Psalidognathus superbus* Fries, including the exerted mandibles of the male, reaches 65 mm. *Acrocinus longimanus* (Linnaeus) has forelegs which, in the male, may surpass 75 mm., with both femora and tibia as long as the body. *Batocera wallacei* Thomson, of New Guinea, may have a body length of 80 mm. with forelegs 85 mm. and antennae in excess of 190 mm.; the Neotropical *Macrodontia cervicornis* (Linnaeus) reaches nearly 170 mm., with mandibles (nearly 60 mm.) longer than the head and thorax combined (310). The Fijian *Xixuthrus heros* (Heer), the Malasian *Xixuthrus microcerus* (White), and the Brazilian *Titanus giganteus* (Linnaeus) reach 135 mm., 120 mm., and 160 mm., respectively.

In form, the majority of species are elongate and often subcylindrical; but those living under bark may be flattened, as *Smodicum,* twig inhabitants may be extremely slender, as *Spalacopsis,* and others may be variously modified in a cryptic manner (see below, p. 122). Cerambycids also exhibit a marked tendency to develop spines, especially on the pronotum, elytra, and appendages, but less commonly on other parts of the body; presumably these have adaptive significance. In some cases they contribute to cryptic form and coloration, but they may have a more significant function in protecting from predators [F. Lane (*in litt.*)].

Sexual dimorphism.—Sexual dimorphism is most commonly reflected in greater length of the male antennae which provides increased sensory area to assist in location of the female, in the relatively larger head, the somewhat narrower elytra, and the smaller, straighter, and less convex abdomen (49). In general, this dimorphism reaches its greatest development and diversity in the Prioninae males (6, 52, 179). The mandibles may be greatly enlarged (e.g., in the African *Jamwonus*) or excessively long and falciform so that they exceed the rest of the head (e.g., in the Neotropical *Macrodontia*). The longer antennae may be serrate, pectinate, imbricate (e.g., in many *Prionus*), or flabellate (e.g., in the Australian *Rhipidocerus* and *Enneaphyllus,* the African *Tereticus*), with the rami very long (e.g., *Closterus*) or biflabellate (e.g., the Neotropical *Sarifer flaviramus* Kirsch). Areas of specialized punctation may be present on various parts of the body (in the male of *Xaurus bennigseni* Lameere such areas are found on the antennae, legs, prothorax, and sternum). Areas of special pubescence or pilosity may exist on the body and appendages (e.g., the abdomen in *Macrotoma,* the prothorax in *Tragosoma,* the tibiae in *Psalidognathus*). The vari-

ous pairs of legs may be of disproportionate length (e.g., *Macrotoma hayesi* Hope) or individual parts of the leg may develop more than the others (as the spined trochanters of some Anoplodermini, the reflexed femora of *Acanthophorus,* the curved tibiae of *Prionoma,* and enlarged tarsi of *Titanus*).

In some of the Anacolini and in *Prionapterus,* the males are smaller than the females and the elytra are abbreviated and dehiscent so that the wings are exposed; the two sexes are so different in form that they have even been described as separate genera (e.g., *Poekilosoma* Serville and *Ceroctenus* Serville). One of the most striking of these, the Columbian *Udeterus brequeti* Thomson, has elytra less than half as long as the body.

Males of *Tillyardia gigas* Carter have the palpi enlarged into open-ribbed, basketlike organs (53) and fly swiftly like cockchafers over dew-covered grasslands on the Dorrigo Plateau of New South Wales (307). In the New Guinean lamiine, *Giganteopalpus helleri* Franz, the male palpi are enormously elongated and slender (118); in the Neotropical *Heteropalpus pretiosus* Buquet, they are branched and hairy. The Micronesian *Acanthosciades* exhibit erect compressed spines on the elytral disc in the male (134). In other Lamiinae, sexual dimorphism is expressed in such features as fringed fore-tarsi in the male and sexual pilosity on the sterna in the Acanthoderini, etc. In the Cerambycinae sexual pubescence is also present on the abdomen of male *Eburodacrys* (229), and the abdomen is fringed in female Obriini and Stenaspini.

Cryptic coloration.—The majority of the adult Cerambycidae are nocturnal or crepuscular in habit, and this fact is usually reflected in their coloration. Those forms which secrete themselves during the day beneath logs, under loose bark, at the base of trees, etc., are commonly dull brownish or black (e.g., most Prioninae, Aseminae, Cerambycini, Phoracanthini). Those which remain inactive and exposed, or become active late in the day are usually cryptically colored, often blending so well with their surroundings as almost to defy detection. Most Lamiinae and some Cerambycinae exhibit this protective or habitat coloration, usually expressed in a mottled appearance resembling the bark of trees. Not uncommonly, especially in tropical forms, elaborate modifications of form, coloration, and pubescence contribute to a marked resemblance to lichens or moss, as the mosslike African *Petrognatha* (193), the American *Desmiphora, Ischnolea,* and *Orteguaze* (184, 229) and the New Zealand *Agapanthida.* This last, together with a moth in the same habitat, is a glaucous green, like the lichens in which it lies concealed (155). Stem-inhabiting species (e.g., *Spalacopsis, Hippopsis*) are frequently much elongated and slender and, when resting flat against the surface of their host plants with their antennae extended forward, are quite inconspicuous. A less slender and flattened twig-infesting species of Japan, *Aulaconotus pachypezoides* Thomson, cuts a groove in the twig the length of its body and lies in it, head downward, with the body closely appressed to the surface (175). Equally remarkable is *Pharsalia saperdoides* Pascoe of

Borneo, which grasps a twig with its front and middle legs and banded antennae; with its body slanting outward and its hind legs drawn in, it resembles a small, dry branchlet (164).

Bright and contrasting coloration.—In marked contrast to the nocturnal Cerambycidae, diurnal forms are generally brilliantly colored, although Paulian states that some shiny metallic Callichromini are strictly nocturnal (131). Among the sun-loving Cerambycinae, most of the Callidiini are metallic blues or greens (as are many Buprestidae with similar habits), the Clytini are brightly and contrastingly colored, and the Stenaspini exhibit a variety of brilliant and resplendent hues. Other groups have bottle-brush-like tufts of hairs on the antennae or legs, the adaptive significance of which is not clear (12). The flower-visiting species of Lepturini and Clytini are commonly marked with yellow and black, corresponding to prevalent flower colors, particularly in the Compositae, as are the North American species of *Crossidius* (211). Perhaps the most striking of these are the bright yellow, pollinose "pollen-beetles" of México (*Ochraethes*) (61). Other diurnal species, the long-legged Indomalasian *Gerania bosci* (Fabricius), for example, have a yellow bloom to the pubescence which disappears in the dead specimen [Gressitt (*in litt.*)].

Among the exceptions to the generally cryptic coloration of the Lamiinae are certain groups with iridescent or metallic structural colors (309), including the species of *Phacellus,* with patches of refractory scales like those of *Morpho* butterflies. In the Tragocephalini and Sternotomini, groups chiefly confined to tropical Africa, nearly all the species are brightly or contrastingly colored (193, 194), and a great many of them are variable. Numerous "varieties," "geographic races," and "aberrations" have been named. The adults are diurnal and fly during the sunny hours of the day but rest on bushes and trees in the afternoon. Similarly, the diurnal *Zygrita diva* Thomson of New Guinea varies from nearly black to red with a wide variety of spotting, banding, and striping [Gressitt (*in litt.*)], as does the Mexican *Essostrutha fimbriolata* Bates [F. Lane (*in litt.*)]. Whether they are distasteful, and thus warningly colored as are the bright red asclepiad-feeding *Tetraopes* of North America (160), remains to be determined. Even *Tetraopes* may not, however, be universally avoided by birds; Robertson (273) states that *Asclepias* plants in his yard were freed of them by the rose-breasted grosbeak and Forbes (116) reported occasional specimens in bluebird stomachs. Among the Prioninae, nocturnal habits and somber coloration are the rule, but a few tropical forms are diurnal and brightly colored (e.g., the Central and South American *Pyrodes* and *Psalidognathus s. str.*) (14). Some species are sexually dichromatic in this regard (most of the Neotropical species of *Meroscelisus* are dull colored in both sexes, but the male of *M. violaceus* Serville has bright, metallic blue elytra) (179), suggesting a difference in the diurnal activity pattern of male and female. By contrast, among the West Indian *Elateropsis* the males are dull and the females often have bands of white pubescence; in the Neotropical *Pyrodes,*

the females are often brilliantly metallic, whereas the males are dull bronze.

Batesian mimicry.—Batesian mimicry, involving departures in form and behavior as well as coloration coinciding with those of such hymenopterous models as ants, bees, or wasps, or beetle models as lycids, cantharids, or chrysomelids, is widespread among the diurnal Cerambycidae, especially in the tropics, and exhibits varying degrees of complexity. Unfortunately, few ecologists have carried out the necessary field studies to permit the proper interpretation of the individual cases occurring locally in a given region. Among the earliest contributions to the subject are those of Bates (13) in the Amazon region; the most extensive information was provided by Shelford (291) in Borneo. Gahan (124) and Linsley (213) also have commented extensively on the subject. Shelford's studies are notable as an intensive analysis of the fauna of a small area 20 miles in diameter in which he encountered more than 70 species of mimetic Cerambycidae. No similar study has been made, to my knowledge, in any other comparable area, nor have Shelford's interpretations been confirmed by re-investigation. Nevertheless, his general conclusions appear to be valid, although details may be subject to later revision.

In its simplest form, Batesian mimicry among the Cerambycidae is expressed as "group mimicry," in which the species in a genus, in related genera, or in a tribe have a general but nonspecific resemblance to those of some other group of insects. Thus, the almost universally flower-visiting Lepturinae exhibit certain group characteristics commonly associated with this habit, (e.g., an elongate and attenuated body form and bright coloration). The same characteristics are common among flower-visiting wasps, and selection for wasplike flight and behavior has occurred repeatedly among Lepturinae in many parts of the world. The Clytini, also group mimics of wasps, generally have short antennae, long legs, and yellow and black coloration. These have been selected for wasplike mannerisms in running about over logs and branches in the sunlight. The closely related Tillomorphini, basically clytine in form, consists of species smaller in size. The genera in this tribe are group mimics of ants and usually exhibit a slight constriction of the elytra at the middle, often accentuated by a transverse ivorylike band or an oblique pubescent line, as well as a swollen, convex, pubescent elytral apex, and different coloration before and after the median constriction. This type of group mimicry, although apparently similar in origin to that of the Lepturinae and Clytini, involves some morphological departure from the ancestral type and is often perfected for models of diverse types (307); it may even vary geographically to coincide with geographical color variation in the model (210). Among other sets of group mimics may be mentioned the broad, flattened, sedentary Pteroplatini, which are mimics of specific lycid beetles throughout tropical America; the ovoid, apterous Parmenini, which resemble spiders in both hemispheres; and the Rhyncophora-like Mesosini and Apomycini of the Old World. The Necydalini, on the other hand, are elongated, with short antennae and abbreviated elytra which ex-

pose the flying wings on the abdomen, and are group mimics of Hymenoptera, including not only wasps and bees but also parasitic forms. Such cerambycids are chosen for wasplike behavior only if they are diurnal in habit. Thus, the small, diurnal *Molorchus* exhibit wasplike mannerisms, but not the similarly formed noctural *Methia.* In the Methiini, only the diurnal *Coleomethia evaniiformis* Knull (172) is mimetic, adopting the behavior of an evaniid wasp.

Among the Lamiinae there are scattered ant mimics which run rapidly and seldom take flight (94), and many of these are wingless (185). On the other hand, the African genus *Neonitocris* (36) contains species which mimic ichneumonids, braconids, and vespids (124). Lycid mimics reappear numerous times in the Cerambycinae, Lepturinae, and Lamiinae (14, 71, 124, 182, 193, 291), and the Brazilian *Sternacanthus undatus* (Olivier) resembles *Erotylus sexfasciatus* Fabricius. Further, the Burmese *Estigmenida variabilis* Gahan mimics the hispid *Estigmena chinensis* Hope, the Malayan *Ixais castelnaudi* (Thomson), the weevil *Episomus chlorostigma* Wiedemann, and members of the Philippine genus *Doliops,* weevils of the genus *Pachyrrhynchus.* In South America, various genera, including the remarkable *Zelliboria* also resemble hispids (181), *Ites,* cassidids (182), and *Probatius choliniformis* Lane, the weevil, *Naedius elegans* Lane & Moure (183). On the other hand, *Compsosoma mutillarium* Klug, together with the similarly hairy and brightly colored weevil *Cyphus myrmosarius* Perty, are striking mimics of a mutillid wasp [F. Lane (*in litt.*)]. In Australia, the excessively slender species of *Lygesis* (307) resemble coreids of the genus *Leptocorisa,* and the remarkable Brazilian *Meltzerella lutzi* Costa Lima (69) is marked and colored like a pyrhocorid or reduviid.

Sexual dimorphism in mimicry occurs in the prionine genus *Anacolus,* in which the males resemble wasps and the females mimic *Chauliognathus* and other cantharids.

From the ecological and evolutionary standpoint one of the most interesting groups of Batesian mimics is the Neotropical Rhinotragini. In this tribe, closely related forms resemble, associate with, and behave like groups of insects which are very diverse in form, coloration, and habits. In fact, most of the genera consist of species which are quite obviously mimetic. Some closely resemble protected groups of beetles, as *Chauliognathus* and other cantharids; others mimic and congregate with lycids or resemble hispid leaf beetles. Genera closely allied to these contain flower-visiting species which are vespiform, and the resemblance of such groups to their individual models in flight behavior is very close (13). Rhinotragini also resemble parasitic Hymenoptera and even mosquitoes. Finally, several groups mimic bees, especially those of the genera *Melipona* and *Megachile* (13). Similar, but less diverse, groups of closely related mimics occur in other sections of the family (213, 291).

Müllerian mimicry.—In the West Indies, a "Müllerian ring" of synaposomatic mimicry involving distasteful or inedible forms of various insect

groups, particularly the Coleoptera and Hemiptera, has been investigated by Darlington (81). Included are lycids (*Thonalmus*) colored bright orange or red with the apex of the elytra blue, which are presumed to be models for numerous Batesian mimics, including cerambycids. In a series of experiments, Darlington attempted to determine the relative edibility of some of these Cerambycidae by means of the lizard, *Anolis sagrei* (Duméril & Bibron). In no case did the lizards eat or show any interest in the *Thonalmus*-like species, although they did eat 11 plainly colored specimens of six rather diverse species (some Cerambycinae, some Lamiinae).

Although experimental evidence is accumulating indicating that bees, wasps, and beetles of the families referred to above are generally avoided by vertebrate predators or have a certain degree of immunity from attack [see especially Shelford (291), Jones (160), and Darlington (81)], it appears significant that bee and wasp mimics of the Cerambycidae are generally flower-frequenting types, and that lycid, cantharid, chrysomelid, and ant mimics are most commonly found on trees and other vegetation in the same situations as their models. Also, although there is an abundance of species of Cerambycidae, individuals are often rare, and in mimetic forms they are usually exceeded numerically by their models. Most objections to the theory of mimicry have been directed at the mechanism by which the mimetic pattern is produced. Nicholson (242) emphasizes that protective resemblance apparently offers a slight advantage to certain individuals, rather than to the species as a whole, and thus has been brought about by a gradual accumulation of slight changes in the direction of an ultimate mimetic pattern.

Aptery and adaptations for subterranean existence.—The Lamiinae contain the greatest diversity of wingless or flightless forms. Many of these have been grouped by Breuning (35) into two artificial and admittedly polyphyletic groups, the "Parmenini" and "Morimopsini," comprising 86 genera and hundreds of species scattered over the major continents and various island groups. However, wingless or flightless forms occur in various other sections of the Lamiinae (37) and in the Prioninae (179), and to a lesser extent in other subfamilies. The condition is commonly associated with subterranean or nocturnal habits but also develops in windswept insular environments (203) and other habitats, such as the crests of high mountains, where sedentary habits favor preservation of species (e.g., *Schreiteria bruchi* Melzer, attached to the peculiar *Azorella yareta* at elevations of 3000 to 4000 m. in the Andean region) (42).

Atrophy of the wings is by far the commonest of the adaptations of adult beetles associated with subterranean (root-feeding) habits. The loss of wings usually involves both sexes in the Lamiinae (35), but only the female among the Lepturinae (for example, Philini, Vesperini, *Apatophysis*) (31). Sometimes the male is larger (as in *Morimus*) (49), or it may be considerably smaller (as in *Moneilema*) (262). In the Prioninae, although both sexes may be apterous (as are the Neotropical *Psalidocoptus, Apterocaulus, Prionocalus, Hypocephalus*), more commonly the female is

wingless or at least flightless. In the Indian *Dorysthenes* and the Neotropical *Hypocephalus armatus* Desmarest, the mandibles are elongate and curve downward from the base; they are more prominent in the male. In the males of *Dorysthenes,* the last segment of the palpi is enlarged and dilated; in the female of *Prionapterus* the palpi of the female are more developed than those of the male. Further, in some of the subterranean species the eyes are reduced in size, sometimes only in the female (e.g., *Meroscelisus*), sometimes in both sexes (e.g., *Hypocephalus*). In males of some of the subterranean Anoplodermini (e.g., *Acanthomigdolus*) the posterior trochanters are prolonged as an extended spine (44).

Perhaps the most extreme case of adult adaptation to burrowing habits is the Brazilian *Hypocephalus armatus* Desmarest. The head is peculiarly shaped, the antennae very short, the prothorax enormous, and the legs adapted for digging (the posterior pair have greatly inflated femora and arched tibia) (5, 129).

Adaptations to root feeding also involve larvae (as in *Vesperus, Acmaeops, Agapanthia*). The adults of *Vesperus,* for example, lay large numbers of eggs (a thousand or more) in irregular groups on or under surface objects, like stones, at the base of trees or vines, or in openings and crevices. The primary larvae are cylindrical and parallel-sided, with long antennae, three ocelli on each side, and very long abdominal setae. After hatching from the egg, they drop to the ground and work their way down through the soil to their food supply, then molt and develop into a short, compressed, posteriorly enlarged, truncate, blind, scarablike grub (276).

The apterous arboreal *Sciadella saltator* Gressitt (134) is not only one of the smallest species in Micronesia (1.8 to 3.3 mm.), but has the habit of jumping several centimeters when disturbed.

Natural Mortality

Mortality in cerambycid populations from biotic and physical causes may be high. Although published quantitative data are relatively few, something is known of factors which, in general, operate to control population levels. For example, over a five-year period, Peterson (253) found 25-per-cent mortality of *Saperda calcarata* Say eggs, which he attributed to "infertility, climatic conditions, and other physical causes," and 18 per-cent loss which he attributed to parasites, especially *Iphiaulax* (Braconidae). Mortality of 29 per cent occurred among first- and second-year larvae as a result of "climatic conditions, unsuitable or insufficient food, predators, and excessive sap flow in spring," and further mortality in the third and fourth years was caused by a complex of hymenopterous parasites, and hibernating larvae and pupae by woodpeckers.

Predators and parasites.—Among the most effective natural enemies of the Cerambycidae and other wood-boring beetles are predaceous Coleoptera (114). Species of *Cucujus* feed upon both the adults and the larvae. The same is true of such ostomids as *Tenebroides* and *Temnochila,* and numerous clerids, particularly *Cymatodera, Thanasimus,* and *Chariessa,*

have similar habits (33, 62). Elaterids of the genera *Alaus, Hemirhipis* and *Stenagostus* also destroy them (98, 114, 131, 281), as do the predaceous larvae of Rhaphidiidae (Neuroptera), which live under bark and prey upon all kinds of wood-boring larvae.

Among the Hemiptera, ambush bugs of the genus *Phymata* include adult cerambycids in their prey (11), and the reduviid *Arilus cristatus* (Hahn) preys on adult *Megacyllene* in North America (279), while *Margasus afziella* Stål and *Rhinocorus nitidulus* (Fabricius) feed on *Neonitocris princeps* Jordan and *Acmocera conjux* Thomson in Africa (141, 161).

Diptera also include several predatory or parasitic groups which feed upon larval cerambycids, the Asilidae and Rhagionidae in particular. Sarcophagids and tachinids parasitize both adult and larval Cerambycidae (105, 147, 195, 278, 296), but their habits are little known. Duffy (93) has also recorded an unknown phorid parasite of *Anelaphus nanus* (Fabricius).

The role of parasitic Hymenoptera in reducing longicorn populations is also poorly understood. Chittenden (63) has recorded a number of parasites of North American species and Elliot & Morley (103) and Kleine (171) have given long lists of parasites reared from various European Cerambycidae. Most of these are in primitive groups (45). Members of the braconid tribes Xordini and Acaenitini are commonly parasitic on economically important species (236), as are several groups of ichneumonids (80, 274, 312), but their significance has not been evaluated. The nematode, *Bradynema strasseni* Wülker, has been reported as a larval parasite of *Rhagium* (268) and adult beetles are commonly infested with mites and pseudoscorpions (308).

Birds—particularly woodpeckers, creepers, flycatchers, nighthawks, vireos, nuthatches, and warblers—derive a part of their food from adult cerambycids (39, 116). Woodpeckers in particular are especially fond of longicorn larvae (40, 41). The sound of larvae at work is frequently discernible at a distance of several feet and the birds probably locate the borers in this manner. Beal (15) found that the wood-boring larvae (including cerambycids) make up 14 per cent of the diet of the downy woodpecker and 77 per cent of the diet of the three-toed woodpecker. According to Jones (160), an analysis of stomach contents of nearly 2000 North American vireos yielded 48 species of Cerambycidae. Among the reptiles, lizards take a large toll of longicorns. They congregate on fallen logs and branches to which the beetles are being attracted and capture them as they fly in. The stomach of the common fence lizard of California (*Sceloporus*) will often yield a dozen or more specimens of *Neoclytus* or *Xylotrechus*. Bats, also, have been observed to capture longicorns, and examination of bat roosts has revealed elytra of *Prionus, Ergates,* and other large night-flying species. Undoubtedly many other insectivorous mammals feed upon longicorns when the opportunity arises.

Man must also be included among the significant vertebrate predators of Cerambycidae. From the time of Pliny, who describes the "cossus," a delicacy highly appreciated by the Romans and now generally believed to

be the larva of *Cerambyx cerdo* Linnaeus (29), writers have recorded the use of cerambycid larvae as human food. Duffy (93) lists 22 species, the larvae of which are eaten in various parts of the world, and other species are referred to by Bodenheimer (29). Most of these are Prioninae and Lamiinae, but a few large Cerambycinae are included. Sometimes they are cooked (22) or specially prepared (257), but more often they are eaten raw. In the New Hebrides, Solomon Islands, and New Guinea, where large prionine larvae are extensively used as food, the populations are kept very low in many places [Gressitt (*in litt.*)]. Adult cerambycids—for example, the Australian *Eurynassa australis* Boisduval—are also eaten occasionally. As might be expected, larvae are used as bait by fresh-water fishermen in various parts of the world (87), and in East China mulberry twigs infested with larval *Ceresium sinicum* White are sold as food for caged birds (132, 175). It is doubtful, though, that these latter types of human predation are important factors in population control.

Diseases.—Apparently little has been recorded of diseases of Cerambycidae. Garman (127) recorded *Beauveria bassiana* (Balsamo) (as *Sporotrichum globuliferum* Spegazzini) from dead adults of *Cyllene robiniae* (Forster) in Kentucky, not recognizing that the fungus attacks living individuals. MacLeod (217) reports the same species from the European *Acanthocinus aedilis* (Linnaeus). Other fungi, supposed to be *Isaria* and *Entomophthora,* have been reported affecting *Phryneta spinator* Fabricius in Africa and *Saperda carcharias* Linnaeus, respectively, in Europe (93). Crawshay (79) refers to an unidentified disease of the larvae of *Tetropium gabrieli* Weise which developed when they were reared under glass. Beeson (20) also noted a disease, supposed to be caused by a virus, in larvae of *Chelosterna scabrator* (Fabricius), which appeared when they were reared in glass tubes (an associated fungus which developed on the larvae was considered to be secondary), and Hasegawa & Koyama (142a) reported a virus disease associated with *Batocera lineolata* Chevrolat. However, the occurrence of virus diseases in Cerambycidae has not been confirmed.

Physical factors.—Temperature is one of the most obvious limiting factors in the life of cerambycid borers, a fact which often accounts for their ecological distribution within a log (130) and has been used as a control measure for injurious species under both natural (74) and artificial (75) conditions. Thus, the exposure of hickory logs to direct sunlight for a few hours will kill all of the immature stages of *Megacyllene caryae* (Gahan) beneath the exposed bark; and in smaller sticks of mesquite two days' exposure will kill 40 per cent of all stages of *Megacyllene antennatus* (White) to a depth of .5 in., a week's exposure will destroy 75 per cent to a depth of .75 in., and two weeks', 90 per cent to the same depth (75). Graham (130) found a wide range in death temperatures for *Monochamus scutellatus* (Say) ranging from 43°C. to 53°C. (average: 47°C.) for adults and from 45°C. to 50°C. (average: 48°C.) for larvae; he attributes their ability to survive and adapt to new conditions to the exceptional individuals falling outside of the average.

However, among Cerambycidae, as with other insects (228), tolerance for high temperatures is greatly influenced by the relative humidity. Savely (281) found that the highest temperatures tolerable to *Romaleum atomarium* (Drury), *Monochamus titillator* (Fabricius), and *Acanthocinus nodosus* (Fabricius) were 49°, 50°, and 46°C., respectively, when exposed in air with a relative humidity of approximately 10 to 15 per cent. With relative humidity of 100 per cent they could tolerate only temperatures of 44°, 44°, and 43°C., respectively. The explanation offered for this phenomenon was that evaporation of water from a larva in dry air has a cooling effect that undoubtedly lowers the body temperature below that of the surroundings. Of the various larvae which Savely tested, those of *Chrysobothris* sp. (Buprestidae) were most resistant to desiccation, and those of *Dendroides bicolor* Newman (Pyrochroidae) least resistant. The percentage of body weight lost by evaporation from larvae of *R. atomarium, M. titillator,* and *A. nodosus* was intermediate between that of *Chrysobothris* and *Dendroides.* These differences in resistance to desiccation are correlated with the conditions of moisture in the logs in which the beetles live. Thus the three Cerambycidae are normally found only where the wood is moist, and the humidity of the air around them is probably always near the saturation point. Furthermore, they are not found in parts of a log where the subcortical temperature is likely to be as high above the air temperature as that in the parts inhabited by *Chrysobothris.*

Dürr (97) made a study of the effect of physical factors on adults of *Hylotrupes bajulus* (Linnaeus) and concluded that adults can live, copulate, and oviposit under extremely variable combinations of constant temperature and relative humidity. They seem to be effected to a greater extent by temperature than by relative humidity. The results obtained at various relative humidities, but at a constant temperature, show no significant variation in the average number of eggs per female, in longevity, or in the percentage of females which oviposited. When a constant humidity and different temperatures were used, however, marked deviations occurred. The effect of temperature is most striking on longevity, number of eggs per female, and the percentage of females which oviposited (females were kept with males constantly). Thus, a relatively cool temperature of 52°F. caused the female to live longer, whereas a fairly high temperature of 98°F. shortened the life of the female considerably. The relatively high temperature (98°F.) caused the female to lay fewer eggs than at room conditions, and the cool temperature (52°F.) wholly prevented oviposition. Possibly copulation was also prevented; none could be observed during the daytime at this temperature.

ACKNOWLEDGMENTS

The helpful suggestions of John A. Chemsak, J. Linsley Gressitt, Paul D. Hurd, Jr., Frederico Lane, and George B. Vogt are gratefully acknowledged.

LITERATURE CITED

1. Adams, C. C., *Bull. Illinois Lab. Nat. Hist.*, **11**, 30–280 (1915)
2. Alexander, R. D., *Ohio J. Sci.*, **57**, 101–13 (1957)
3. Anderson, T. J., *Ann. Rept. Dept. Agr. Kenya, 1929*, 433–463 (1930)
4. Anon., *J. Wash. Acad. Sci.*, **47**, 339 (1957)
5. Araujo, R. L., *Rev. brasil. entomol.*, **2**, 175–92 (1954)
6. Arrow, G. J., *Proc. Zool. Soc. London*, 113–16 (1944)
7. Aurivillius, C., *Coleopterorum Catalogus*, **39**, 1–574; **73**, 1–704 (1912, 1923)
8. Axelrod, D. I., *Evolution*, **2**, 127–44 (1948)
9. Ayontantis, A. J., Kortzas, C. B., and Pelecassis, E. D., *Ann. Inst. Phytopathol. Benaki*, **7**, 11–14 (1953)
10. Back, E. A., *J. Econ. Entomol.*, **11**, 411–14 (1918)
11. Balduf, W. V., *Can. Entomologist*, **71**, 66–74 (1939); *Bull. Brooklyn Entomol. Soc.*, **35**, 161–69 (1940)
12. Bates, H. W., *The Naturalist on the River Amazons* (Murray, London, England, 2 vols., 1863)
13. Bates, H. W., *Trans. Roy. Entomol. Soc. (London)*, 243–55 (1870)
14. Bates, H. W., *Biol. Centrali-Americana, Coleopt.*, **5**, 1–436 (1879–85)
15. Beal, F. E. L., *U. S. Dept. Agr., Biol. Survey Bull. No. 37* (1911)
16. Becker, G., *Naturwisschenschaften*, **26**, 462–63 (1938); **30**, 253–56 (1942)
17. Becker, G., *Z. angew. Entomol.*, **30**, 104–18 (1943)
18. Becker, G., *Z. angew. Entomol.*, **37**, 1–28 (1955)
19. Beeson, C. F. C., *Nature*, **126**, 112 (1930)
20. Beeson, C. F. C., *Indian Forest Records, Entomol.*, **16**, 1–16 (1931)
21. Beeson, C. F. C., and Bhatia, B. M., *Indian Forest Records, Entomol.*, **5**, 1–235 (1939)
22. Bequaert, J., *Am. Museum Nat. Hist. J.*, **21**, 191–200 (1921)
23. Beutenmuller, W., *J. N. Y. Entomol. Soc.*, **4**, 73–81 (1896)
24. Blackman, M. W., and Stage, H. H., *N. Y. State Coll. Forestry Syracuse Univ. Tech. Publ.*, **10**, 11–115 (1918); **24**, 3–269 (1924)
25. Blackwelder, R. E., *U. S. Natl. Museum Bull.*, **185**, 551–627 (1948)
26. Blair, K. G., *Entomologist's Monthly Mag.*, **73**, 261–68 (1937)
27. Blatchley, W. S., *Can. Entomologist*, **52**, 42–46, 68–72 (1920)
28. Bodenheimer, F. S., *Die Schädlingsfauna Palästinas* (P. Parey, Berlin, Germany, 438 pp., 1930)
29. Bodenheimer, F. S., *Insects as Human Food; A Chapter of the Ecology of Man* (W. Junk, The Hague, Netherlands, 352 pp., 1951)
30. Bondar, G., *Agronomia (Rio de Janeiro)*, **12**, 29–31 (1953)
31. Boppe, P., *Genera Insectorum*, **178**, 1–121 (1921)
32. Bosc, J. M., *Ing. agron. (Buenos Aires)*, **4**, 49–63 (1942)
33. Boving, A. G., and Champlain, A. B., *Proc. U. S. Natl. Museum*, **57**, 575–649 (1920)
34. Box, H. E., *List of Sugar Cane Insects* (Commonwealth Institute of Entomology, London, England, 101 pp., 1953)
35. Breuning, S., *Longicornia*, **1**, 29–262 (1950)
36. Breuning, S., *Ann. musée roy. Congo Belge, Sér. in 8°, Sci. Zool.*, **4**, 1–46 (1950)
37. Breuning, S., *Rev. franç. Entomol.*, **22**, 119–23 (1955)
38. Breuning, S., *Entomol. Arb. Museum G. Frey*, **7**, 1–199 (1956)
39. Brooks, F. E., *U. S. Dept. Agr. Bull.*, **262**, 1–7 (1915)

40. Brooks, F. E., *U. S. Dept. Agr. Bull.*, **847**, 1–42; **886**, 1–12 (1920)
41. Brooks, F. E., *J. Agr. Research*, **26**, 313–17 (1923)
42. Bruch, C., *Physis (Soc. entomol., Paris)*, **11**, 361–65 (1935)
43. Bruch, C., *Notas Museum La Plata, Zool.*, **5**, 118 (1940)
44. Bruch, C., *Notas Museum La Plata, Zool.*, **6**, 163–71, 355–69 (1941)
45. Brues, C. T., *Psyche*, **34**, 73–90 (1927)
46. Buchka, E., *Entomol. Blätter*, **38**, 133 (1942)
47. Buchner, P., *Holznährung und Symbiose* (J. Springer, Berlin, Germany, 64 pp., 1928)
48. Buchner, P., *Endosymbiose der Tiere mit Pflanzlichen Mikroorganismen* (Birkhäuser, Basel, Switzerland, 771 pp., 1953)
49. Bugnion, E., *Bull. biol. France et Belg.*, **65**, 234–65 (1931)
50. Burke, H. E., *J. Econ. Entomol.*, **14**, 450–52 (1921)
51. Butovitsch, H. von, *Entomol. Tidskr.*, **60**, 206–58 (1939)
52. Campos, F., *Rev. col. nacl. Rocafuerte*, **9**, 1–25 (1927)
53. Carter, H. J., *Proc. Linnean Soc. N. S. Wales*, **37**, 480–91 (1913)
54. Champion, H. C., *Entomologist's Monthly Mag.*, **55**, 219–24 (1919)
55. Champlain, A. B., and Kirk, H. B., *Entomol. News*, **37**, 288–91 (1926)
56. Champlain, A. B., and Knull, J. N., *Entomol. News*, **43**, 253–57 (1932)
57. Chang, S. C., *J. Agr. Forestry (Taiwan)*, **3**, 55–75 (1954)
58. Cheesman, E., *Hunting Insects in the South Seas* (Allan & Co., London, England, 243 pp., 1932)
59. Chemsak, J. A., *Pan-Pacific Entomologist*, **34**, 42 (1958)
60. Cheron, G., *Le Coléopteriste*, **2**, 24–26 (1890)
61. Chevrolat, L. A. A., *Ann. soc. entomol. France*, [3]**8**, 451–504 (1860)
62. Chittenden, F. H., *Entomol. Am.*, **6**, 154–55 (1890)
63. Chittenden, F. H., *Proc. Entomol. Soc. Wash.*, **3**, 95–102 (1894)
64. Chittenden, F. H., and Linell, M. L., *Proc. Entomol. Soc. Wash.*, **4**, 42–43 (1896)
65. Clausen, C. P., *U. S. Dept. Agr. Circ. No. 168* (1931)
66. Clausen, C. P., *U. S. Dept. Agr. Circ. No. 266* (1933)
67. Cleare, L. D., *Agr. J. Brit. Guiana*, **4**, 82–90 (1931)
68. Corella, L. B., *Bol. agr. territ. españ. Golfo de Guinea (Madrid)*, 39–91 (1942)
69. Costa Lima, A. da, *Rev. Entomol.*, **1**, 139–42 (1931)
70. Costa Lima, A. da, *Terceiro catalogo dos insectos* (Escola nacional de agronomía, Rio de Janeiro, Brazil, 460 pp., 1936)
71. Costa Lima, A. da, *Insectos do Brazil*, **8**(2), **9**(3) (Escola Nacional de Agronomía, Rio de Janeiro, Brazil, 1953–55)
72. Cottier, W., *New Zealand J. Agr.*, **57**, 28–29 (1938)
73. Craighead, F. C., *U. S. Dept. Agr., Secy's. Rept. No. 107*, 1–24 (1915)
74. Craighead, F. C., *Proc. Entomol. Soc. Wash.*, **22**, 106–8 (1920)
75. Craighead, F. C., *J. Forestry*, **19**, 250–54 (1921)
76. Craighead, F. C., *J. Agr. Research*, **22**, 189–220 (1921)
77. Craighead, F. C., *Can. Dept. Agr. Bull. No. 27*, 1–239 (1923)
78. Craighead, F. C., *U. S. Dept. Agr. Misc. Publ. No. 657*, 1–679 (1950)
79. Crawshay, G. A., *Trans. Roy. Entomol. Soc. (London)*, 183–212 (1907)
80. Cushman, R. A., and Rohwer, S. A., *Proc. U. S. Natl. Museum*, **57**, 503–22 (1920)
81. Darlington, P. J., *Trans. Roy. Entomol. Soc. (London)*, **87**, 681–96 (1938)
82. Darlington, P. J., *Zoogeography* (Wiley, New York, N.Y. 672 pp., 1957)
83. Derksen, W., *Z. Morphol. Ökol. Tiere*, **37**, 683–734 (1941)

84. Deschamps, P., *Bull. soc. entomol. France,* **49,** 104–10 (1945)
85. Deschamps, P., *Ann. sci. nat. Zool. et biol. animale,* **15,** 449–533 (1953)
86. Deshpande, V. G., and Karandakar, K. R., *J. Univ. Bombay,* [B]**16**(5), 1–14 (1948)
87. Distant, W. L., *Insecta Tranvaaliensia,* **I,** 299 pp. (1904)
88. Dodd, A. P., *The Biological Campaign Against the Prickly-Pear* (Commonwealth Prickly Pear Board, Brisbane, Queensland, 177 pp., 1940)
89. Dodd, A. P., *Proc. Hawaiian Entomol. Soc.,* **14,** 336 (1952)
90. Downes, J. A., and Williams, D., *Colonial Plant and Animal Prods.,* **1,** 33–51 (1950)
91. Dudich, E., *Entomol. Blätter,* **16,** 146–61; **17,** 136–40, 145–55 (1920–21)
92. Duffy, E. A. J., *Proc. S. London Entomol. Nat. Hist. Soc., 1947–48,* 82–110 (1949)
93. Duffy, E. A. J., *A Monograph of the Immature Stages of British . . . Timber Beetles* (British Museum of Natural History, London, England, 292 pp., 1953)
94. Duffy, E. A. J., *A Monograph of the Immature Stages of African Timber Beetles* (British Museum of Natural History, London, England, 338 pp., 1957)
95. Dumbleton, L. J., *New Zealand J. Agr.,* **55,** 295–98 (1937)
96. Dürr, H. J. R., *J. Entomol. Soc. S. Africa,* **15,** 83–89 (1952)
97. Dürr, H. J. R., *Union S. Africa Dept. Agr., Bull. No. 337,* 1–78 (1954); *Union S. Africa Dept. Agr., Entomol. Mem.,* **4,** 1–136 (1956)
98. Dusham, E. H., *Cornell Univ. Agr. Expt. Sta. Bull. No. 407,* 171–203 (1921)
99. Dutt, N., *Bull. Entomol. Research,* **47,** 777–83 (1956)
100. Eaton, C. B., and Lyon, R. L., *Calif. Forest Range Expt. Sta. Tech. Paper No. 11,* 1–11 (1955)
101. Eidmann, H., *Mem. Akad. deut. Forstwiss.,* **1,** 240–71 (1943)
102. Ekblom, T., *Skand. Arch. Physiol.,* **61,** 35–48 (1931)
103. Elliott, E. A., and C. Morley, *Trans. Roy. Entomol. Soc. (London),* **7–75** (1907)
104. Ellis, H. W., *Proc. Roy. Entomol. Soc. (London),* **1,** 54 (1927)
105. Emden, F. I. van, *Entomologist's Monthly Mag.,* **86,** 182–206 (1950)
106. Escherich, K., *Die Forstinsekten Mitteleuropas,* **2** (P. Parey, Berlin, Germany 663 pp., 1923)
107. Faber, A., *Jahrb. Ver vaterl. Naturk. Württemburg,* **108,** 71–73 (1953)
108. Falck, R., *Cellulosechemie,* **11,** 89–91 (1930)
109. Felt, E. P., *N. Y. State Museum Mem.,* **8,** (1905–6)
110. Felt, E. P., and Joutel, L. H., *N. Y. State Museum Bull. No. 74,* 1–86 (1904)
111. Fisher, W. S., *Proc. Entomol. Soc. Wash.,* **17,** 77–79 (1915)
112. Fisher, W. S., *Proc. Entomol. Soc. Wash.,* **19,** 173–74 (1917)
113. Fisher, W. S., *Rev. chilena hist. nat.,* **40,** 324–26 (1936)
114. Fiske, W. F., *Proc. Entomol. Soc. Wash.,* **9,** 23–27 (1907)
115. Fonseca, J. P. da, *Rev. Entomol.,* **1,** 37–41 (1931)
116. Forbes, S. A., *Bull. Illinois Lab. Nat. Hist.,* **4,** 80–148 (1880)
117. Franssen, C. J. H., and Tiggelovend, L. M. J., *Entomol. Mededeel. Ned.-Indië,* **4,** 55–57 (1937)
118. Franz, E., *Senckenbergiana,* **34,** 37–39 (1953)
119. Froggatt, W. W., *Agr. Gaz. N. S. Wales, Misc. Publ. No. 2075,* 261–67 (1919)
120. Fullaway, D. T., *J. Econ. Entomol.,* **47,** 696–700 (1954)
121. Fuller, C., *Agr. J. Union S. Africa,* **8,** 240–42 (1914)

122. Gahan, C. J., *Ann. Mag. Nat. Hist.*, [6]**13,** 520–21 (1894)
123. Gahan, C. J., *Trans. Roy. Entomol. Soc. (London)*, 433–52 (1900)
124. Gahan, C. J., *Proc. S. London Entomol. Nat. Hist. Soc., 1912-13,* 28–38 (1913)
125. Gardiner, L. M., *Can. Entomologist,* **86,** 465–70 (1954)
126. Gardiner, L. M., *Can. Dept. Agr., Bi-Monthly Rept. Forest Biol. Div.,* **13,** 2 (1957)
127. Garman, H., *Kentucky Agr. Expt. Sta. Univ. Kentucky, Bull. No. 231,* 3–22 (1921)
128. Gerberg, E. J., *J. Econ. Entomol.* **44,** 317 (1951)
129. Gounelle, E., *Ann. soc. entomol. France,* **74,** 105–8 (1905)
130. Graham, S. A., *Ecology,* **6,** 397–411 (1925)
131. Grandi, G., *Introduzione allo studio dell' entomologia,* **2** (Edizione Agricole, Bologna, Italy, 1332 pp., 1951)
132. Gressitt, J. L., *Lingnan Nat. Hist. Survey, Spec. Publ. No. 1,* 1–60 (1942)
133. Gressitt, J. L., *Longicornia,* **2,** 1–667 (1951)
134. Gressitt, J. L., *Insects of Micronesia,* **17,** 62–183 (Bishop Museum, Honolulu, T. H., 1956)
135. Gressitt, J. L., *Ann. Rev. Entomol.,* **3,** 207–30 (1958)
136. Grösswald, K., *Nachrbl. deuts. Pflanzenschutzdienst (Berlin)*, **19,** 17–19 (1939)
137. Gunn, D., *Dept. Agr. Pretoria (Div. Entomol.) Bull.,* **8,** 1–8 (1916)
138. Gunn, D., *Dept. Agr. S. Africa, Bull. No. 6,* 1–22 (1919)
139. Haliburton, W., *Can. Entomologist,* **83,** 36–38 (1951)
140. Hamilton, J., *Can. Entomologist,* **16,** 35–38 (1884)
141. Hancock, G. L. R., *Ann. Rept. Dept. Agr. Uganda, 1925,* 25–28 (1926)
142. Hardouin, R., *Bull. soc. entomol. France,* **50,** 58–60 (1945)
142a. Hasegawa, K., and Koyama, R., *Intern. Union Forest Research Organizations, 12th Congress* (Oxford, England, 3 pp., Mimeo., 1956)
143. Heintze, A., *Entomol. Tidskr.,* **46,** 21–34 (1925)
144. Heitz, E., *Z. Morphol. Ökol. Tiere, Abt. Wiss. Biol.,* **7,** 279–305 (1927)
145. Herrick, G. W., *J. Econ. Entomol.,* **16,** 546–48 (1923)
146. Herrick, G. W., *Insect Enemies of Shade Trees* (Comstock Co., Ithaca, N.Y., 417 pp., 1935)
147. Hess, A. D., *New York State Agr. Expt. Sta. (Geneva, N.Y.) Bull. No. 688,* 3–93 (1940)
148. Hoffman, W. E., *Lingnan Sci. J.,* **1,** 37–59 (1934)
149. Hollande, A. C., *Ann. univ. Grénoble,* **21,** 459–517 (1909)
150. Hopkins, A. D., *U. S. Dept. Agr., Progr. Work 1917,* 353 (1916)
151. Horn, O., *Gesammelte Abhandl. Kenntniss Kohle,* **10,** 23–38 (1930)
152. Horton, J. R., *J. Agr. Research,* **11,** 371–82 (1917)
153. Houlbert, C., *Insecta,* **2,** 302–10 (1912)
154. Hubbard, H. G., *Insects Affecting the Orange* (U. S. Department of Agriculture, Division of Entomology, Washington, D. C., 227 pp., 1885)
155. Hudson, G. V., *New Zealand Beetles and Their Larvae* (Ferguson & Osborne, Ltd., Wellington, New Zealand, 236 pp., 1934)
156. Hungerford, H. B., *J. Econ. Entomol.* **32,** 596 (1939)
157. Husain, M. A., and Khan, A. W., *Indian J. Agr. Sci.,* **10,** 945–59 (1941)
158. Ingles, L. G., *J. Entomol. and Zool.,* **25,** 57–59 (1933)
159. Janjua, N. A., and Mehra, R. N., *Bull. Entomol. Research,* **40,** 203–6 (1949)
160. Jones, F. M., *Trans. Roy. Entomol. Soc. (London)*, **80,** 345–86 (1932)
161. Jover, H., *Rev. pathol. végétale et entomol. agr. France,* **32,** 258–75 (1954)

162. Kalshoven, L. G. E., *Landbouw*, **12**, 165–90 (1936)
163. Kalshoven, L. G. E., *De Plagen van de Cultuurgewassen in Indonesie* (W. Van Hoeve, 's-Gravenhage, Netherlands, 2 vols., 1065 pp., 1951)
164. Kalshoven, L. G. E., *Entomol. Ber.*, **15**, 438–40, 528–33 (1955)
165. Kaszab, Z., *Festschr. Embrik Strand*, **4**, 149–63 (1938)
166. Keen, F. P., *U. S. Dept. Agr. Misc. Publ. No. 273*, 1–280 (1952)
167. Kemner, N. A., *Entomol. Avdel.*, **26**, 1–8 (1918)
168. Kemner, N. A., *Entomol. Tidskr.*, **43**, 81–138 (1922)
169. Kimmey, J. W., and Furniss, R. L., *U. S. Dept. Agr. Tech. Bull. No. 851*, 1–61 (1943)
170. King, J. L., *J. Econ. Entomol.*, **13**, 432–33 (1920)
171. Kleine, R., *Entomol. Blätter*, **5**, 177–79, 207–12 (1909)
172. Knull, J. N., *Ohio J. Sci.*, **37**, 301–8 (1937)
173. Knull, J. N., *Ohio State Univ. Studies, Ohio Biol. Survey, Bull. No. 39*, 133–354 (1946)
174. Knuth, P., *Handbuch der Blutenbiologie* (W. Engelmann, Leipzig, Germany, 2 vols., 1802 pp., 1898–99)
175. Kojima, T., *J. Coll. Agr. Imp. Univ. Tokyo*, **10**, 101–28; **11**, 263–308 (1929–31)
176. Krogerus, R., *Notulae Entomol.*, **7**, 121–26 (1927)
177. Kunhi Kannan, K., *Dept. Agr. Mysore, Entomol. Ser. Bull. No. 8*, 1–24 (1928)
178. Lameere, A., *Coleopterorum Catalogus*, **52**, 1–108 (1913)
179. Lameere, A., *Bull. sci. France et Belg.*, **49**, 1–14 (1915)
180. Lane, F., *O Democratica*, **1404**, 1 (1944)
181. Lane, F., *Dusenia*, **2**, 1–19 (1951)
182. Lane, F., *Rev. brasil. entomol.*, **1**, 195–201 (1954)
183. Lane, F., *Dusenia*, **7**, 1–32 (1956)
184. Lane, F., *Am. Museum Novitates*, **1889**, 1–17 (1958)
185. Lea, A. M., *Agr. Gaz. Tasmania*, **9**, 221 (1902)
186. LeConte, J. L., *Smithsonian Inst. Publ., Misc. Collections*, **265**, 279–348 (1873)
187. Leech, H. B., *Pan-Pacific Entomologist*, **14**, 69 (1938)
188. Leech, H. B., *Pan-Pacific Entomologist*, **20**, 30 (1944)
189. Leech, H. B., *Can. Entomologist*, **76**, 211 (1944)
190. Lepesme, P., *Les insectes des palmiers* (P. Leschevalier, Paris, France, 903 pp., 1947)
191. Lepesme, P., *Longicornia*, **1**, 587–89 (1950)
192. Lepesme, P., *Longicornia*, **3**, 771–73 (1958)
193. Lepesme, P., and Breuning, S., *Longicornia*, **1**, 423–506 (1950)
194. Lepesme, P., and Breuning, S., *Longicornia*, **3**, 269–347 (1958)
195. Lepesme, P., and Paulian, R., *Rev. zool. botan. africaines*, **37**, 89–92 (1943)
196. Lepesme, P., and Villiers, A., *Trav. sect. tech. agr. trop.*, **1**, 27–70 (1944)
197. Lesne, P., *Rev. botan. appl. et agr. coloniale*, **10**, 781–91 (1930)
198. Lieu, K. O. V., *Florida Entomologist*, **27**, 62–101 (1945)
199. Lieu, K. O. V., *Notes entomol. chinoise (Musée Heude)*, **11**, 69–119 (1947)
200. Lindroth, C. H., *Götesborgs Kgl. Vetenskapsakad.-Vitterhetssamhälles Handl.* [B]4(3), 1–911 (1949)
201. Lindroth, C. H., *The Faunal Connections Between Europe and North America* (Wiley, New York, N.Y., 344 pp., 1957)
202. Linell, M. L., *Entomol. Am.*, **5**, 39–40 (1889)
203. Linsley, E. G., *Pan-Pacific Entomologist*, **11**, 72–74 (1935)

204. Linsley, E. G., *Pan-Pacific Entomologist*, **12**, 119, 199–200 (1936)
205. Linsley, E. G., *Pan-Pacific Entomologist*, **14**, 177 (1938)
206. Linsley, E. G., *Proc. Pacific Sci. Congr., Pacific Sci. Assoc., 6th Meeting*, **4**, 269–82 (1939)
207. Linsley, E. G., *Entomol. News*, **51**, 285–90 (1940)
208. Linsley, E. G., *J. Econ. Entomol.*, **33**, 561–63 (1940)
209. Linsley, E. G., *Proc. N. Engl. Zool. Club*, **21**, 17–42 (1942)
210. Linsley, E. G., *Proc. Calif. Acad. Sci.*, [4]**24**, 21–96 (1942)
211. Linsley, E. G., *J. Kansas Entomol. Soc.*, **30**, 81–89 (1957)
212. Linsley, E. G., *Pan-Pacific Entomologist*, **34**, 105–24 (1958)
213. Linsley, E. G., *Ann. Entomol. Soc. Am.* (In press)
214. Linsley, E. G., in *Symposia on Zoogeography* (American Association for the Advancement of Science, Washington, D. C., In press)
215. Lovell, J. H., *Psyche*, **22**, 67–84, 109–17 (1915)
216. Lundberg, S., *Entomol. Tidskr.*, **77**, 176–78 (1957)
217. MacLeod, D. M., *Can. J. Botany*, **32**, 818–90 (1954)
218. MacSwain, J. W., *Pan-Pacific Entomologist*, **34**, 104 (1958)
219. Mansour, K., and Mansour-Bek, J. J., *J. Exptl. Biol.*, **11**, 243–56 (1934)
220. Mansour, K., and Mansour-Bek, J. J., *Enzymologia*, **4**, 1–6 (1937)
221. Marcu, O., *Zool. Anz.*, **92**, 65–66 (1930)
222. Marcu, O., *Bull. sect. sci. acad. roumaine*, **15**, 240–45 (1932)
223. Maxwell-Lefroy, H., *Indian Insect Life* (Thacker, Spink & Co., Calcutta, India, 786 pp., 1909)
224. May, A. W. S., *Queensland Agr. J.*, **6**, 137–50 (1946)
225. Mayet, V., *Les insectes de la vigne* (C. Coulet, Montpellier, France, 470 pp., 1890)
226. Mayr, E., *Systematics and the Origin of Species* (Columbia University Press, New York, N. Y., 334 pp., 1942)
227. McKeown, K. C., *Australian Museum Mem.*, **10**, 1–190 (1947)
228. Mellanby, K., *J. Exptl. Biol.*, **9**, 222 (1932)
229. Melzer, J., *Archiv. inst. biol. (Brasil)*, **1**, 143–58 (1928)
230. Mendizabal, M., *Bol. patol. vegetal y entomol. agr. Madrid*, **11**, 387–410 (1943)
231. Miller, J. M., *U. S. Dept. Agr. Bur. Entomol. Plant Quarantine, E599*, 1–9 (1943)
232. Miller, N. C. E., *Malay Agr. J.*, **22**, 367–68 (1934)
233. Mjoeberg, E., *Arkiv. Zool.*, **2**, 21–86 (1905)
234. Morgan, A. C., *U. S. Dept. Agr. Bur. Entomol. Bull. No. 63*, 63–66 (1907)
235. Morgan, C. V. G., *Proc. Entomol. Soc. Brit. Columbia*, **44**, 28–30 (1948)
236. Muesebeck, C. F. W., and Walkley, L. M., *U. S. Dept. Agr. Monograph No. 2*, 90–184 (1951)
237. Müller, H., *Die Befruchtung der Blumen durch Insekten* (W. Engelmann, Leipzig, Germany, 478 pp., 1873)
238. Müller, W., *Arch. Mikrobiol.*, **5**, 84–147 (1934)
239. Murthi, B. K., *J. Mysore Agr. Exptl. Union*, **4**, 69–75 (1931)
240. Neander, A., *Entomol. Tidskr.*, **49**, 202–8 (1928)
241. Nguyên-Cong-Tiêu, *Bull. Econ. Indochine*, **198**, 735–44 (1928)
242. Nicholson, A. J., *Australian Zoologist*, **5**, 10–104 (1927)
243. Niesiolowski, W., *Polskie Pismo Entomol.*, **18**, 221–24 (1949)
244. Olliff, A. S., *Agr. Gaz. N. S. Wales*, **3**, 895–97 (1892)
245. Paillot, A., *Ann. Epiphyt.*, **19**, 369–79 (1934)

246. Palm, T., *Medd. Statens Skogsforsk.*, **40,** 3–242 (1951)
247. Palm, T., *Entomol. Tidskr.*, **77,** 158–63 (1957)
248. Paramanow, S., *Z. angew. Entomol.*, **35,** 63–81 (1953)
249. Park, O., *Ann. Entomol. Soc. Am.*, **23,** 57–80 (1930)
250. Parkin, E. A., *J. Exptl. Biol.*, **17,** 364–77 (1940)
251. Paulian, R., *Mem. inst. sci. Madagascar,* [E]**1,** 1–22 (1952)
252. Pechuman, L. L., *Bull. Brooklyn Entomol. Soc.*, **35,** 113–16 (1940)
253. Peterson, L. O. T., *Ann. Rept. Entomol. Soc. Ontario 1947,* 56–61 (1948)
254. Pettey, F. M., *Farming in S. Africa, Rept. No. 6,* 1–3 (1946)
255. Pettey, F. M., *Union S. Africa, Dept. Agr. Sci. Bull. No. 340,* 1–36 (1953)
256. Peyerimhoff, P. de, *Ann. soc. entomol. France,* **101,** 359–408 (1933)
257. Planet, L., *Naturaliste,* **3,** 119 (1889)
258. Plavilstshikov, N. N., *Leningrad, Inst. zool. Acad. Sci. U.R.S.S.*, **21,** 1–611 (1936)
259. Pollard, D. G.: *Bull. Entomol. Research.*, **45,** 553–61 (1954)
260. Porter, C. E., *Rev. chilena hist. nat.*, **25,** 495–501 (1921)
261. Pruthi, H. S., and Mani, M. S., *Imp. Council Agr. Research Sci. Monographs,* **16,** 1–42 (1945)
262. Psota, F. J., *Coleopterological Contribs.*, **1,** 111–41 (1930)
263. Ramachandran, S., *Indian J. Entomol.*, **14,** 214 (1953)
264. Regnier, R., *Bull. soc. sci. nat. Rouen,* **6**(59), 53–55 (1925)
265. Rehman, K. A., *Proc. Indian Sci. Congr., 29th Meeting,* **3,** 174–75 (1943)
266. Rehman, K. A., *Punjab Fruit J.*, **10,** 141–44 (1946)
267. Reid, J., *Can. Dept. Agr. Ann. Rept. Forest Insect and Disease Survey, 1957,* 49–51 (1958)
268. Reineck, G., *Deut. Entomol. Z., 1919,* Suppl., 1–92 (1919)
269. Reymond, A., *Longicornia,* **1,** 563–76 (1950)
270. Richards, O. W., *J. Ecol.*, **14,** 244–81 (1926)
271. Ripper, W., *Z. Vergleich. Physiol.*, **13,** 314–33 (1930)
272. Risbec, J., *Faune colonies franç.*, **6,** 1–214 (1937)
273. Robertson, C., *Trans. St. Louis Acad. Sci.*, **5,** 569–98 (1891)
274. Rohwer, S. A., *Proc. U. S. Natl. Museum,* **57,** 405–74 (1920)
275. Ruggles, A. G., *J. Econ. Entomol.*, **8,** 79–85 (1915)
276. Ruiz Castro, A., *Fauna entomolgica de la vid en España, IV: Coleoptera* (Instituto Español de Entomologica, Madrid, Spain, 133 pp., 1947)
277. Rungs, C., *Bull. soc. sci. nat. et phys. Maroc,* **17,** 13–14 (1937)
278. Saalas, U., *Ann. Entomol. Fennici,* **9,** 23–28 (1943)
279. Sanborn, C. E., and Painter, H. R., *Oklahoma Agr. Exp. Sta. Bull. No. 113,* 1–8 (1917)
280. Saraiva, A. C., *J. Entomol. Soc. S. Africa,* **2,** 101–14 (1939)
281. Savely, H. E., *Ecol. Monographs,* **9,** 323–85 (1939)
282. Schiödte, J. C., *Naturhist. Tidsskr.*, **10,** 369–458 (1863)
283. Schomann, H., *Z. Morphol. Ökol. Tiere,* **32,** 542–612 (1937)
284. Schuch, K., *Z. Pflanzenkrankh. u. Pflanzendienst,* **47,** 572–85 (1937)
285. Schwarz, E. A., *Proc. Entomol. Soc. Wash.*, **6,** 21–22 (1904)
286. Schwitzgebel, R. B., and Wilbur, D. A., *J. Kansas Entomol. Soc.*, **15,** 37–44 (1942)
287. Setty, H. T. R., and Rao, H. H., *Current Sci. (India)*, **22,** 308–9 (1953)
288. Shafik, M., *Egypt, Ministry Agr. Tech. Bull. No. 182,* 1–9 (1928)
289. Sharma, H. N., and Singh, B. N., *Bull. United Prov. Dept. Agr.*, **21,** 1–8 (1940)

290. Sharp, D., *The Cambridge Natural History*, **6**, 285–88 (Macmillan, London and New York, 1918)
291. Shelford, R., *Proc. Zool. Soc. London*, 230–84 (1902)
292. Shelford, V. E., *Animal Communities in Temperate America as Illustrated in the Chicago Region* (University of Chicago Press, Chicago, Ill., 367 pp., 1937)
293. Shiraki, T., *Catalogue of Injurious Insects of Japan* (Natural Resources Division, Headquarters, Commander Allied Powers, Tokyo, Japan, 4 vols., 1952)
294. Shu-Chen, C., *J. Agr. Forestry*, **1**, 127–32 (1952)
295. Smirnov, D. A., *Trav. soc. nat. St. Petersbourg, sect. zool.*, **40**, 1–15 (1911)
296. Spencer, G. J., and Buckell, E. R., *Proc. Entomol. Soc. Brit. Columbia*, **54**, 29–36 (1957)
297. Stace-Smith, G., *Proc. Entomol. Soc. Brit. Columbia*, **42**, 28 (1946)
298. Stebbing, E. P., *Indian Forest Insects of Economic Importance—Coleoptera* (Eyre & Spottiswoode, London, England, 648 pp., 1914)
299. Steinhaus, E. A., *Insect Microbiology* (Comstock Co., Ithaca, N.Y., 763 pp., 1946)
300. Steinhaus, E. A., *Principles of Insect Pathology* (McGraw-Hill, New York, N.Y., 757 pp., 1949)
301. Swezey, O. H., *Proc. Hawaiian Entomol. Soc.*, **7**, 16 (1928)
302. Swezey, O. H., *Proc. Hawaiian Entomol. Soc.*, **12**, 343–403 (1945)
303. Swezey, O. H., *Bishop Museum Spec. Publ. No. 44*, 1–266 (1954)
304. Swezey, O. H., *Pan-Pacific Entomologist*, **31**, 37–39 (1955)
305. Taylor, H. S., *New Zealand J. Agr.*, **94**, 357–58 (1957)
306. Thorpe, W. H., *Biol. Revs. Cambridge Phil. Soc.*, **5**, 177–212 (1930)
307. Tillyard, R. J., *The Insects of Australia and New Zealand* (Angus & Robertson, Ltd., Sydney, Australia, 560 pp., 1926)
308. Tippmann, F. F., *Z. Wiener entomol. Ges.*, **34**, 154–57 (1949)
309. Tippmann, F. F., *Entomol. Blätter*, **49**, 148–53 (1953)
310. Tippmann, F. F., *Dusenia*, **4**, 181–228 (1953)
311. Tippmann, F. F., *Entomol. Blätter*, **50**, 175–83 (1954)
312. Townes, H., and Townes, M., *U. S. Dept. Agr. Monograph No. 2*, 184–409 (1951)
313. Trägårdh, I., *Bull. Entomol. Research*, **21**, 1–8 (1930)
314. Uvarov, B. P., *Soc. Biogeograph. Mem. No. 6*, 231–73 (1938)
315. Vayssière, P., *Bull. soc. entomol. France*, **40**, 160–62 (1935)
316. Villiers, A., *Coléoptères cerambycides de l'Afrique du nord* (P. Lechevalier, Paris, France, 152 pp., 1946)
317. Walsh, B. D., *Proc. Entomol. Soc. Philadelphia*, **3**, 403–30; **5**, 194–215 (1864–65)
318. Waterston, J. M., *Bull. Bermuda Dept. Agr.*, **9**, 52–53 (1940)
319. Weber, P. W., *Proc. Hawaiian Entomol. Soc.*, **14**, 218, 327–30; **15**, 127–30; **16**, 162–64 (1951–56)
320. Webster, F. M., *Bull. Illinois Lab. Nat. Hist.*, **7**, 1–14 (1904)
321. Wiedner, H., *Z. Pflanzenkrankh. u. Pflanzendienst*, **46**, 305–26 (1936)
322. Wolcott, G. N., *J. Dept. Agr. Porto Rico*, **7**, 5–313 (1923)
323. Zimmerman, E. C., *Insects of Hawaii*, **1** (University of Hawaii Press, Honolulu, T.H., 206 pp., 1948)

BIOLOGY OF APHIDS[1]

By J. S. Kennedy[2]
Agricultural Research Council Unit of Insect Physiology, Zoology Department, Cambridge, England

AND

H. L. G. Stroyan[2]
Ministry of Agriculture and Fisheries Plant Pathology Laboratory, Milton Road, Harpenden, Hertfordshire, England

Börner & Heinze have provided a full modern account (10) of the superfamily Aphidoidea, and we have used their family group names. Bodenheimer & Swirski's recent book (8) on aphids of the Middle East devotes several chapters to general biology, while remarking with very good reason: "The student of aphids is requested never to generalize." We have tried to select topics and recent (preferably experimental) pieces of work contributing to the general frame of reference of the subject, although we have been unable to give adequate space to needed qualifications and often were ignorant as to how representative the few species studied may be. The preponderance of work hitherto has been on aphids in temperate regions or belonging to the predominantly temperate Aphididae. Inevitably, we have perpetuated this bias, while recognizing the lacuna concerning aphid ecology in the moist tropics.

The largest family of aphids today, the Aphididae, have achieved their success evolutionarily, and as agricultural pests, through parasitic exploitation of the temperate flora. The members of this flora make highly inconstant hosts, with marked seasonal cycles and a great diversity of growth patterns in the rather long cool summer. The unique feature of aphids is that they have as a group developed a specialized, typically parasitic, "vegetative" mode of life without being committed to it, combining with it more normal locomotory, reproductive, and overwintering capacities within the one species. The combination is effected through an elaborate system of polymorphism in which the alternative forms differ in the same way as the successive juvenile and adult forms of Holometabola.

[1] The survey of the literature pertaining to this review was completed in March, 1958, but included a number of still unpublished papers. We are much indebted to the authors who kindly lent us their manuscripts, without which this article would have been badly out of date. With equal sincerity we apologize to those authors whose names should, on the basis of priority, have been mentioned but instead must be found only in the more recent papers and reviews that limited space has permitted us to cite.

[2] We are indebted to M. J. Way and A. D. Lees for criticism of the population and form-determination sections, respectively, and to D. Hille Ris Lambers for painstaking criticism of the whole manuscript.

Parasitic Life on the Plant

Parasitic "degeneration."—The specialized host-exploiting form is an obligate parasite: the familiar, typically apterous, viviparous, and parthenogenetic female reproducing its like ("virginopara"). It evolved very early in the history of the group, and the variant of it known as the fundatrix, which hatches from the fertilized egg and exploits the spring flush of growth in the host, still presents more or less obviously the greatest degree of parasitic modification (75). The modifications are of the usual two kinds—reduction of sensorimotor functions and hypertrophy of growth and reproductive functions—but aphids combine these in a distinctive way. The adult looks and lives like the juvenile stages, but these are never obligatorily sessile, and the adult is still the most active stage (except in a few specialized cases) as it is in free-living insects. In this way the aphids keep up (to an extent varying among aphids and plants) with the constantly shifting most-favoured feeding sites as plant development proceeds, or disperse from overcrowded positions to new ones on the same or neighbouring plants [Ito (33, 34)]. This retention, in nearly all aphids, of sensorimotor powers greater than those retained by most coccids and many other parasites, has accompanied not lesser but greater parasitic specialization in other respects. Female-producing parthenogenesis is frequent among parasitic insects, occurring in Homoptera among Coccoidea and Aleyrodidae as well as aphids. But while oviparity is the rule among Coccoidea and is retained by Adelgidae and Phylloxeridae among Aphidoidea, an extreme form of viviparity prevails in most aphids. Ovulation is a juvenile more than an adult function, and embryonic development of her young begins before the mother's birth, in the grandmother's body (56). The food intake of one individual sustains three generations. Postembryonic development is correspondingly rapid, and so is larviposition, which begins soon after the last moult as the sole reproductive function reserved to the adult. Individual fecundity of the virginopara is modest, 100 offspring being quite a high total. It is the telescoping of the generations which gives aphids their unequalled rates of multiplication and the oft-quoted astronomical totals of theoretical progeny from one female in one year—such as 524 billion for *Aphis fabae* Scopoli (10, 62, 80).

Feeding.—The basis for this extraordinary productivity is a method of feeding shared by many Auchenorrhyncha and Coccoidea: tapping the plant's own nutrient sap stream in the phloem sieve tubes. In a turgid plant this sap is under pressure, and when the rostra of feeding *A. fabae*, *Myzus persicae* (Sulzer), and *Tuberolachnus salignus* (Gmelin) were severed, sap was observed to exude from the stylet stumps left in the plant (49, 96). Mittler (66) found that the rate of exudation was not appreciably less than the rate of honeydew output by intact *T. salignus* feeding on the same willow stems. "Sucking insects" thus appears to be a misnomer for most aphids and to imply an underestimation of their parasitic status. A cell tapped by *T. salignus* is refilled by its neighbours

approximately 100,000 times per hr. (66). The work done by this remarkable and little-understood pump in the plant accounts for the very high rates of food ingestion recorded for some aphids compared with some of the most voracious of chewing insects (Table I).

The flow and the sugar and nitrogen contents of the sap from cut stylets of *T. salignus* are maintained for hours and even days (66, 67, 101). From this evidence, still limited to one species, it appears that, by acting as an adventitious "sink" for water and solutes, the insect harnesses the plant's natural translocatory mechanism and is thereby fed chemically, as well as physically. Just how much of the exploitation of and damage to plants is achieved by this simple indirect means calls for further study. For by this means alone aphids feeding in sufficient numbers are in a position to cause breakdown and release into the sap stream of material from tissues not directly attacked, i.e., to induce premature senescence and improve the plant temporarily as a food source, in addition to the typical stunting of growth. Many species do also inject salivary toxins causing

TABLE I

SOME RATES OF FOOD INGESTION BY LEAF-CHEWING INSECT LARVAE AND AN APTEROUS VIVIPAROUS APHID (*T. salignus*), EXPRESSED AS THE WEIGHT RATIO, FOOD/INSECT

Insect	Intake in 24 hr.			Authority
	Fresh food/ Fresh insect	Dry food/ Dry insect	Nitrogen/ Dry insect	
Pieris brassicae (Linnaeus): Instar IV	2.0	1.2	0.006	Evans (21)
Bombyx mori (Linnaeus): Instar V	0.8	1.5	0.006	Legay (57) & Hiratsuka (32)
Schistocerca gregaria Forskål: Instars I & V	1.0	—	—	Davey (14)
Tuberolachnus salignus (Gmelin): Instar I	31.8	12.8	0.16	Mittler (65, 67, 69)†
Instar IV	10.6	3.1	0.04	
Adult aptera	7.9*	2.2	0.03	

* Similar rates have been recorded for *Myzus persicae* (Sulzer) (16), much lower ones for *Acyrthosiphon pisum* (Harris) (3) and *Brevicoryne brassicae* Linnaeus (16); the differences could be in the plants or aphids.

† 0.1 Per cent nitrogen in the fresh sap is assumed for these particular specimens on a tree with sensecing foliage, although twice as much was recorded at bud-burst (67). Dry matter is taken as 8 per cent of fresh sap (67).

histological damage and local yellowing in certain host plants (10, 12, 85), but it is questionable whether a cell which is currently feeding sap into the stylet tips piercing it is so damaged, and salivation is most copious where no actual feeding occurs (66, 95). Toxic effects grade off in one sense toward local necrosis amounting to a self-defence mechanism of the plant [e.g., *Viteus vitifoliae* (Fitch) on some resistant vines (10)], but also in the opposite sense toward pronounced acceleration of the normal processes by which the plant pours its substance down the aphid's throat. A few specimens of the prolific and devastating *Therioaphis maculata* (Buckton) on susceptible American alfalfa varieties induce a pathological "vein-clearing" senescence in leaves growing above those fed upon, as well as diffuse local yellowing near the feeding site (85). This is not a virus infection, although virus-disease symptoms also often resemble premature senescence and in a few cases have been shown to improve the plant as a host for the aphid vector (26, 47).

The aphid, in any case, controls the rate of sap intake. If a feeding *T. salignus* is anaesthetized, it does not become distended with sap although the sap will flow out of the stylets as soon as they are cut (66); *A. fabae* feeds (6) and gains weight (19) faster when attended than when unattended by ants; and different strains of *Rhopalosiphum maidis* (Fitch) take up sap at different rates from the same sorghum (84). Evidently the aphids do not always feed at the maximum rate the plant would allow, and the key problem is the sensory control. Small honeydew output is not in itself, therefore, evidence of feeding in parenchyma instead of sieve tubes (8). *Melaphis* Walsh (Fordinae), *Aspidaphium* Börner (Aphididae), and a few other genera live on mosses, but we are aware of no evidence as to whether they feed in generalized leaf cells or in the primitive conducting elements.

No satisfactory artificial feeding technique is yet available, despite many published and unpublished attempts. Van Hoof (95) has taken the logical step of applying pressure (6 to 12 atm.) to fluid behind a membrane but has not demonstrated any faster uptake than has been obtained before without pressure (60, 98, 16): much less than the normal feeding rate.

A minority of Aphidoidea, especially in the ancient families Thelaxidae, Pemphigidae, Adelgidae, and Phylloxeridae, inject into the growing organs of their primary hosts certain substances that induce extra local growth, forming crude or elaborate galls (10, 106). Nuorteva (81) summarizes his own and other evidence that the substances in the saliva of aphids and other Homoptera and Heteroptera may be growth hormones and enzymes drawn previously out of the plant. Intriguing new experiments by Anders (2) suggest that the gall-forming substances injected by the vine phylloxera *V. vitifoliae* may be simply amino acids. Quite apart from providing shelter, the blister galls formed by *Cryptomyzus ribis* (Linnaeus) on *Ribes* leaves are physiologically more favourable to the aphid than ungalled areas of the same leaves, for they shorten the normal unfavourable phase of leaf ma-

turity (49). Anders (1) finds that for certain vines the "tolerance" toward *V. vitifoliae* resulting from the galling response is a more effective resistance mechanism than local necrosis. In such cases the galled tissue shows de-differentiation (1, 2), and the aphids appear to be feeding at a terminal "sink" instead of in a translocatory pipe line. How different the feeding and nutritional physiology of these minority groups may be can be judged from the extreme case of the Phylloxeridae, in which even among non-gall-makers the hind-gut is closed.

Nutrition.—*T. salignus* on willow stems [Mittler (67, 68)] again is the only case for which the composition of the sap actually ingested is known and may be compared with the honeydew, which in itself many workers have analyzed. But Lindemann's (58) results from *C. ribis* and sap obtained by exudation or expression from the *Ribes* twigs bearing the infested leaves are in good agreement with Mittler's. The sap from cut stylets of *T. salignus* contained 5 to 10 per cent sugar (usually sucrose only, and forming at least 90 per cent of the dry matter), and up to 0.2 per cent nitrogen in the form of amino acids and amides but no larger molecules [but see Auclair (3)]. Although there is more nitrogen in whole leaf tissue so that a sap feeder must, as long supposed, feed faster to equal the nitrogen intake of a leaf eater, sap intake can, in fact, be so fast that the aphid's intake of organic nitrogen can exceed that of leaf-eating insects (Table I). Whether the aphid assimilates a higher proportion of the ingested nitrogen (at least 55 per cent in *T. salignus*) than other insects is uncertain, but it does appear to use more (effectively all in *T. salignus*) of what it assimilates for growth (68). The rest is excreted apparently unchanged. Auclair's report (3) that the haemolymph and honeydew of *Acyrthosiphon pisum* (Harris) contain similar concentrations of amino nitrogen suggests that absorption from the gut may be by passive diffusion. As to excretion of metabolic nitrogen, Lamb (54) reported free ammonia in considerable quantities in fresh honeydew of *A. fabae* and pointed out that for excretory purposes honeydew-producing aphids were in much the same position as aquatic animals, which are ammonotelic.

Unlike the amino nitrogen, nearly all the carbohydrate ingested is excreted by *T. salignus* (67), some in the ingested form of sucrose or derived monosaccharides. But a large part is converted in this and other aphids and coccids to a trisaccharide, melezitose, or gluco-sucrose (59, 97). Since phloem-tapping aphids do, in effect, "filter out" nitrogenous matter while rejecting carbohydrate (although only Lachnidae and a very few Aphididae possess a filter chamber) a possible role for the trisaccharides is suggested by Treherne's work (93) on the opposite process of absorption of carbohydrates in *Schistocerca gregaria* Forskål (Orthoptera, Acrididae). There, absorption through the gut wall is apparently by passive diffusion which is facilitated by active conversion of the smaller molecules to trehalose in the haemolymph. Reciprocally, conversion to melezitose or gluco-

sucrose within the aphid's gut could be a mechanism impeding absorption of sugars. If filter chambers do, in fact, serve to impede sugar absorption, they may only be needed when sap passage is slow. The available information on digestive enzymes in aphids is given by Waterhouse (97), Nuorteva (81), and in Börner & Heinze (10). Older reports of starch and protein splitting enzymes in saliva or gut have not been confirmed in aphids, but the discovery of a salivary pectinase in some forms of some species [McAllan & Cameron (63)] recalls many reports of intercellular penetration by the stylets in the middle lamella (10).

The excretion of unabsorbed amino acids might suggest that something else must be in short supply in the sap to account for the wasteful method of feeding by phloem-tapping aphids (104). But this inference becomes implausible when the rapid flow of sap costs almost nothing and permits higher rates of assimilation than other feeding methods, with important morphological and physiological economies in digestion, excretion, and water conservation. The real problem for sap feeders, even given a rapid intake, is vitamin deficiency [Koch (52)], but with aphids less attention has been paid to the likely role of symbiotes in this context than to their role in meeting a supposed nitrogen dificiency. The possibility remains that one function of symbiotes is to convert excretory nitrogen back into usable form for the aphid (86).

Field populations.—That which in nature prevents actual realization of the vast reproductive potential of aphids is often said to be restraint by natural enemies. These certainly do exist in great variety and sometimes in great numbers (10), no doubt because aphids are exceptionally rapid converters of plant into animal food and are more or less sessile and exposed, although they are by no means defenseless (16a) and certain species obtain some protection and direct physiological assistance from ants (5, 6, 10, 19) or galls. Most of the numerous studies on populations of economic aphids have accordingly been concentrated on natural enemies, especially insect parasites and predators, which at first sight appear to be acting in the density-dependent manner assumed by prevailing population theory to be necessary for effective control [but see Milne (64)]. However, in only two published cases known to us, *Adelges piceae* (Ratzenburg) [Karafiat & Franz (45)] and *Eriosoma lanigerum* (Hausmann) [Evenhuis (22)], is there quantitative information on the real effect of the insect enemies that has been obtained with check cages from which the enemies were excluded. Both these species are bark feeders, and we still lack such information for any Aphididae, although the work of Way & Banks (100) on *A. fabae* should soon provide some.

How ineffective the combined onslaught of all immediate mortality factors can be against a shoot feeder was brought out by B. D. Smith (88) with *Acyrthosiphon spartii* (Koch). At the time when the adults were being killed off, mainly by birds, after an average of only one day's reproduction (against several weeks on protected plants), the population curve was ris-

ing at its steepest. This brings home, also, the crucial importance of generation time rather than fecundity in aphids: a slight lengthening of the former could tip the balance decisively in favour of the enemies and give the false impression that they alone caused the ensuing decline. The production of winged migrants is a further main cause of local decline and escape from natural enemies; and some authorities (8, 10) hold that the slower-breeding enemies must usually fail to prevent infestations from rising to economic levels. However, Way (99) points out that, in view of the wide dispersal of aphid migrants, population regulation must be considered on a regional rather than a local scale. After multiplying on previous infestations, mobile predators such as coccinellids can concentrate to check the rise of a new one elsewhere, and cultural measures can materially assist this kind of biological control (17, 61, 100). It is when *A. fabae* becomes scarce, because of other factors, that its insect predators seem to have their important effect on subsequent peaks [Way & Banks (100)].

Because of the close parasitic dependence of aphids on very inconstant host plants, the condition of the plants is likely to be particularly important in aphid population dynamics. There is copious, if as yet largely unanalyzed, evidence that aphid numbers depend closely through nutrition, behaviour and form determination, on the state of the plant (8, 9, 11, 49, 62, 90). Density-dependent host factors such as damage to the plant and overcrowding often play a part (5, 9, 34) but are by no means the only ones exerting regular control, for seasonal changes in the plants, not to mention weather, do this anyway [Bodenheimer & Swirski (8)]. Much circumstantial evidence from the reactions of the aphids points to nutritional deficiency in the absence of young growing or senescing organs on the plant (49). Direct evidence of this in terms of sap composition (nitrogen content) is available, again, only for *T. salignus* and *C. ribis* on *Salix* and *Ribes* respectively, where the growth rates, sizes, and birth rates of the aphids show a corresponding depression when leaf growth slows until senescence sets in (67, 58). There may also be a quantitative sap deficiency when low pressure or high viscosity reduces sap intake. Water shortage in the plant, which causes exudation to cease from cut stylets of *T. salignus*, provokes in this and other species various negative responses such as restlessness, slower reproduction, or the production of winged forms; although it can in some circumstances have positive effects (49, 53, 62, 66). A very large proportion of aphid host plants are in any case short-lived (ephemerals, annuals, or biennials), and sap flow ceases more or less completely in all outdoor plants in the temperate zone winter.

The extensive work on aphid resistance in crop plants, reviewed by Painter (82, 83), Müller (78), Anders (1), and Auclair (4), also points to the key role of the host. Painter (83) gives 25 cases of useful resistance in the plant, far more than are known for any other group of insects. He considers that the reason cannot lie in the close parasitic relationship between aphids and their host plants, because resistance is no more likely when the host range is narrow than when it is wide. A more plausible alternative inter-

pretation of these facts would be that resistance commonly depends more on physiological than on taxonomic characters of the plant (11, 12, 27, 91).

Nonparasitic Phases

Forms and functions.—Because the exceptionally favourable food situation for sap feeders is short-lived in any plant, the success of aphids depends on the timely production of forms less degenerate than the apterous vivipara. Species in which the latter form is present (the vast majority) nearly always produce alate viviparae also, even if rarely; and a majority of species can produce a full series of forms. One or both of two principal features distinguish the less degenerate forms: flight and sex (amphigony) with oviparity. Flight effects transfer to fresh hosts on which fully parasitic existence can be resumed either immediately (most alate virginoparae) or in the following spring (alate sexuparae, gynoparae, and males; alate sexual females existing only in a few monoecious Thelaxidae, Callaphididae and Greenideinae). The sexual forms, if any, initiate the physically resistant over-wintering phase of the cycle by laying eggs which enter diapause. But many species overwinter as viviparae, and in some Adelgidae and *Brachycaudus helichrysi* (Kaltenbach) in Britain the hibernating stage is a larva hatching directly from the egg. Amphigony may also occur in the summer in some species which remain on the woody host instead of migrating to herbs [e.g., *Dysaphis devecta* (Walker) and *Aphis farinosa* Gmelin], when some others show reproductive depression [e.g., *Drepanosiphum platanoides* (Schrank)]; *Periphyllus* van der Hoeven partially aestivates as first instar sexuparae. In the host-alternating Fordinae, sexuparae and sexuales are produced and the overwintering eggs are laid in early spring, and egg diapause lasts nearly a year.

The alate virginoparae, sexuparae, and sexuales represent steps back from the fully parasitic, neotenous, paedogenetic state toward a normal insect life history. Most insects exhibit a division of labour between the more or less sedentary juvenile, devoted to feeding and growth, and the active, reproductively specialized adult; the division of labour among the alternative forms of aphids is of the same kind. The juvenile assimilatory functions are most highly developed and sensorimotor functions most reduced in the adult (as well as larval) apterous vivipara. The nonassimilatory, sensorimotor functions of food finding (migration), mating, and oviposition are elaborated in the winged and sexual forms, and only here does the division of labour within one life history become marked. Wigglesworth's thesis (105) that successive and alternative polymorphism are but variations upon a single theme, in the context of developmental physiology, is seen to apply equally to general physiology, behaviour, and ecology in aphids, as already noted in locusts (48).

In general entomology the reproductive and sensorimotor features of adults are customarily bracketed and used indiscriminately as criteria of adulthood. But in alternative polymorphism it is seen that reproduction in

its quantitative, growth, or assimilatory aspect (measured by fecundity, generation time, etc.) is inversely correlated with the elaboration of adult sensorimotor functions. The most juvenile aphid form is the most reproductive (and the most precociously reproductive); there is a reduction of multiplicative capacity accompanying the polarization of reproductive functions toward the last instar in the more adult forms. The Pemphigidae present the extreme in this respect. The migrant vivipara (sexupara) does not feed after arriving on the primary woody host, and its bisexual offspring neither feed nor grow while moulting three or four times, each female producing one single egg. Some reduction of assimilatory functions has been observed in other alatae and sexuales where such information has been sought—as lengthened generation time, reduced fecundity and longevity, or stronger plant-taxonomic and weaker plant-physiological host preferences (8, 9, 10, 51, 62, 68)—although between apterous and alate vivipara the differences in these respects are relatively small, especially when wing muscle autolysis occurs in the young alata. The fundamental division of labour in successive and alternative polymorphism alike appears not to be between assimilation (growth) and reproduction, but between assimilation and sensorimotor functions (48). With Wellington (102), we suspect that some degree of alternative functional polymorphism on similar lines, but without the obvious structural differences which have brought it to light in aphids and locusts, may be found in other insects and may be important in their population dynamics.

The voluminous work on form determination has been catalogued many times (8, 9, 10, 11). As in other insects, short days and low temperatures stimulate the switch to overwintering egg production in some species (55, 56), but the external factors governing presence or absence of wings can still be stated only in vague ecological terms. Form determination is a physiological problem, and much of the current confusion stems from an ecological approach to it: populations rather than individuals are studied, and external factors are assumed to act directly to change form [as in L. M. Smith's (89) ingenious theory of "relative starvation"] rather than to function as quite specific "signal" stimuli triggering a less specific metamorphic mechanism which may have much in common with that operating in ordinary successive polymorphism. Thus, brief fasts by the mother favour wing development in the offspring of various Aphididae including *Megoura viciae* Buckton, but continued malnutrition of that species does not (56). Bonnemaison's (9) "group effect" might also amount to partial mutual inhibition of feeding, since ants which accelerate feeding in *A. fabae* (6) also oppose wing development in the offspring (20). Lees's recent work on *M. viciae* (56) underlines the need to separate experimentally the two stages in the form-changing response: first in the mother stimulated by the environment, and second in her oocytes or embryos; in fact more than two generation are often involved. A possible clue to the second-stage mechanism in the offspring is the discovery that endoparasitic hymenopterous larvae tend

to oppose wing development in *Aphis craccivora* Koch [B. Johnson (39)]; the same reaction was noted also in *Dysaphis bonomii* (Hille Ris Lambers) (H.L.G. Stroyan, unpublished material).

Migration.—More is known about migration in alate viviparous Aphididae than in any other insects, except perhaps army ants and locusts. Even within that one form, aphids exhibit every stage of differentiation of the feature distinguishing all migrants: a transient accentuation of locomotor functions with depression of vegetative functions, such that the insect now travels, by what method being a secondary matter. This differentiation is least in the Callaphididae so far studied [Haine (23, 24)]. Most information is available on the summer alatae of *D. platanoides,* which remain able to fly till near the end of their lives, are late to start larviposition, but continue to produce at a low rate and rarely move as far as the next tree (23). In the Lachnidae and Aphididae studied by B. Johnson (37), locomotor and reproductive activities are more segregated and both intensified. Flight is now confined to the first days of adult life, during which embryo growth is arrested; afterwards, the flight muscles degenerate. A crude series can be traced from the Lachnidae and *M. viciae,* in which larviposition begins only after the loss of wing muscles several days after the last moult, up to *B. brassicae* and *A. fabae,* in which the new alata already contains a complete set of nearly full-grown embryos and can begin depositing them in a matter of hours while still capable of further flight. But at first there is an inhibition of feeding and parturition even on the best host plants, which may last for several days if not actively broken [Moericke (71), B. Johnson (40), Kennedy (50)]. Instead, the antagonistic locomotor reflexes are now highly excitable, so that in appropriate physical conditions the alata takes off to light and rises out of the slow-moving air among plants into the larger-scale air movements above (71, 92). This breakaway is presumably facilitated by the temporary suppression of taking off by gusts of wind, gusts which might otherwise carry the aphid no further than the next plant. The strength of wind required to hold down the fresh alata for a given time increases through a series of species from *D. platanoides* up to *A. fabae* (23, 24). C. G. Johnson (41) has shown conclusively that most of the aphid dispersal in a season occurs when the mean wind speed near the ground is above the supposed 5 m.p.h. limit embedded in textbooks.

The migration of aphids, as of locusts (92), thus begins with the insect actively launching itself into a situation where it is passively transported. The biologically important contrast between this and the station-keeping behaviour of nonmigrant aphids and other insects, seems to be underrated by C. G. Johnson (43) and Taylor (92). Once air-borne, the body hangs nearly vertical and the long wings beat nearly horizontally (36); almost all the work apparently goes into lift, and rates of climb and horizontal air speeds are low. From several years' work with suction and drift traps, C. G. Johnson and Taylor (43, 92) have shown that during those hours of the day when maximum numbers are flying, the shape of the vertical gradient of

their density from 10 up to 4000 feet can be expressed quite precisely in terms of particle diffusion theory and, moreover, can be correlated with the state of the atmosphere, especially its vertical stability as indicated by the temperature gradient. Current atmospheric conditions are not the main variable governing the total numbers flying, as used to be supposed. The main variable, as worked out in detail for *A. fabae,* is the number available to take off, determined ultimately by the conditions causing winged forms to develop and more immediately by their temperature-controlled rate of development and by light (44, 77). Kept between flights on a host plant, they can fly on several successive days (37), but the inferences to be drawn from the hourly trap records are, that while some may do this and others remain aloft at night, yet the overwhelming majority in the field have come to the end of their flying life in a few hours and are probably dead in a few hours more (92).

Passive transport is interrupted as actively as it was initiated, and well before total exhaustion. There is first a transition from the travelling or migratory type of flight to "alighting flight" [Moericke (71)]. In the field this has been described by many workers as flying about near the ground more or less horizontally, resisting drift on the gentler air currents by orienting against them, and alighting actively on plants—all of which contrasts with the fast, obstacle-avoiding flight toward the open sky when aphids first take off. Moericke (72) observed that low-flying aphids often turn away from a white surface, and pointed out that this means a reversal of the earlier orientation toward white and specifically blue-ultraviolet; this, he thought, might be what initiated the descent from the upper air (doubtless dependent partly on air circulation) and then continued to keep the aphids flying low. Unpublished observations in a laboratory "flight chamber" (50) tend to confirm this hypothesis. The actual alighting response (a still unanalyzed compound of arrest and orientation) is elicited by orange-yellow-green, especially yellow, as Moericke's (71, 74) numerous trap experiments have shown. His earlier laboratory work (70), on the allied probing response of *M. persicae* receiving the flight-inhibiting stimulus of contact with a solid surface, showed that yellow was here too the most powerful positive stimulus and blue complementary, as in some other insects and man.

Several workers have demonstrated in the laboratory that the longer the locomotory period beforehand (walking, wing-beating on a pin, flying freely), the stronger are the aphid's opposite "vegetative" responses on alightment: probing, settling to feed, larviposition, resumption of embryogenesis and, finally, wing-muscle histolysis (71, 40, 50). But the first three of these changes are readily reversible, even after a long flight, if the host is not highly suitable, and in the field too the great majority of alighters take off again even from suitable host plants (79). Such re-take-offs by *A. fabae* may be as vigorous as the first ascent or more so (40, 50), and the whole sequence from climbing take-off to horizontal alightment may be repeated many times in the laboratory using a moderately or entirely unsuit-

able plant, to culminate in a final series of weak brief flights to total exhaustion (50). This work indicates that the process by which host responsiveness is induced by locomotion is not an internal unfolding of moods, nor simply a matter of fatigue of some kind, as has been thought (71, 40), but a central nervous process analogous to "successive induction" of antagonistic reflexes in the mammal. Indeed, such a process was implicit in Moericke's discovery (70) of successive colour contrast in *M. persicae*. It seems possible that yellow, which most readily elicits alighting and probing by many aphids, serves to distinguish leaves from sky (with its strongly antagonistic blue-violet component) more clearly than green.

There has been a running controversy between workers studying aphid dispersal from a source crop and up in the air, mainly by means of suction and drift traps [C. G. Johnson (41, 42)], and those, using mainly yellow traps to study low-flying aphids away from the source, who have stressed effects of weather directly on activity [Müller & Unger (76, 94)]. Our impression is that both sides have made valid criticisms of the other's assumptions, and the main trouble is the limitations of traps. Yellow traps cannot measure behaviour independently of aerial population and vertical diffusion, neither of which depends on weather-governed activity. Suction traps measure population and diffusion but mask behaviour, for the catch must dwindle as flights shorten even though take-off frequency may actually be increasing. The case against any important influence of weather on flight activity has rested on the observation that first take-offs are rarely limited by weather, and on the assumption that subsequent nonmigratory flights from plant to plant, although governed by weather, amount to but a small fraction of first take-offs (41). The recent evidence supports an amended version of the old "flight-activity hypothesis", viz., that plant-visiting flights far outnumber first flights and so determine how efficient host finding and virus spreading by the given aerial population will be.

When the weather favours high long-range dispersal, fewer plants may be visited before exhaustion but a wider area is sampled, and such flights cannot be disregarded as without biological or agricultural significance (8, 11, 38, 42). Where, as in the Mississippi Valley of North America, winds favour northward dispersal in spring and southward in autumn, such long traverses may enable species like *R. maidis* to find a succession of host plants suitable for viviparous forms all the year round. But in any agriculturally developed region the crop-infesting aphids which do not undergo maximum dispersal and have more time for host finding will presumably do better. It is noteworthy that high long-range dispersal is more likely in the disturbed air conditions of summer than in spring and autumn (42), and the summer migrants of *A. fabae* and *B. brassicae* are not more, but less, ready to take off in wind than the spring and autumn migrants (24).

Host finding and selection.—The notorious efficiency with which host plants are discovered by Aphididae depends on a typically parasitic, "degenerate" mechanism: random dispersal and alightment by great numbers of

frenetically active migrants, most of which die in the process. Although long inferred from the antennal sensoria of alatae and their arrival from downwind of plants (8), olfactory attraction from a distance has not yet been proved. Alightments by the autumn migrants of *M. persicae* and summer migrants of *A. fabae* proved to be indiscriminate on good and bad hosts (46, 79) [Wertheim believes this to be untrue of fordine sexuparae (103)]. The visual alighting response (discussed above) also seems to be unspecific within wide limits [Moericke (71 to 74)]. Field traps have revealed some differences in the spectral responses of aphid species (and seasonal forms) (71 to 74, 18, 25): certain species colonizing Gramineae and Cyperaceae are markedly less responsive than other aphids to saturated yellow, and *Hyalopterus pruni* (Geoffroy) responds best when some ultraviolet re-emission is added (73). But disappointingly, no correlated differences in spectral re-emission curves from appropriate host leaves have yet been traced (73). Since young and senescing leaves are yellower than mature ones, it is possible that the special effectiveness of yellows in causing alighting and probing may have more to do with the nutritional and physiological status of hosts, than with their taxonomic status. The relative importance of those two aspects of plants in feeding and parturition reactions has been shown to vary among certain species and forms of aphids (51); and *M. persicae,* which in those reactions is one of the least discriminating of aphids plant-taxonomically and most discriminating plant-physiologically, also shows the strongest alighting response to a "pure" yellow (74). But the trap results remain largely uninterpretable pending experimental analysis of the visual alighting responses.

Although it seems clear that host selection occurs mainly after alightment, practically nothing is known, except in the visual probing response, of the stimuli or receptors then involved. The epipharyngeal "gustatory organ" has long been assumed to monitor sap composition and Davidson's early experiments (15) showed that a change in sap composition could make feeding aphids withdraw. The responses of feeding aphids to water and nitrogen deficiency might also be stimulated by physical or chemical properties of the sap. But part of the process of host discrimination occurs without sap uptake, as when alighting aphids make probes lasting less than a minute during which the stylets are typically confined to cell walls and air spaces [Müller (79), van Hoof (95)]. Even with the stylets amputated, *A. fabae* is said to differentiate leaves by age and kind (35). Likely sense organs exist on antennae, labium, and tarsi, and a search for chemical stimulants on the external plant surface is indicated. A beginning has been made by B. D. Smith (88), who extracted the alkaloid sparteine from broom [*Sarothamnus scoparius* (Linnaeus)] colonized by *Acyrthosiphon spartii* (Koch), and induced these aphids to stay longer on pea and bean plants painted with the extract than on similar untreated plants. Sparteine could be regarded as a non-nutritious, plant-taxonomic "token" stimulus.

The existence of a separate class of stimuli betokening sap nutritious-

ness and provided by the sap or physiologically associated factors was postulated in the dual discrimination theory of Kennedy & Booth (49). Yellow reflection seems to be one such. That nutrients could themselves appear on the plant surface as a token of sap nutritiousness was demonstrated in principle by Chibnall (13), who obtained visible deposits of glutamine on ryegrass leaves after soil dressing with ammonium sulphate. But the dual discrimination theory was based on purely circumstantial evidence from the distributions, feeding preferences, and reproductive rates of *A. fabae* and *M. persicae* on leaves of different ages and kinds, and it met with considerable scepticism (59). Better evidence for it has since been forthcoming (49), but still more is needed. In fact, direct experimental analysis of the whole of this key subject of host selection is overdue.

Evolution of Host Relations

Summarizing his studies over 30 years, Mordvilko (75) discussed the way in which he believed aphid life-cycles, and in particular those including heteroecy (regular host alternation within the cycle from one fertilized egg to the next), to have evolved. He postulated a very general tendency for winged aphids to wander from the original hosts on which their species evolved and thereby fortuitiously to encounter other, more recently evolved, plants on which from time to time new cycles of generations would begin, thus leading to a degree of polyphagy. But he supposed that in many cases such new cycles of generations would be limited by the inability of either sexual forms or fundatrices to live on the new host, these forms being more closely bound to the original host. In such a situation, selection would operate in favour of any lineages in which winged aphids returned, equally fortuitously, to the original host before giving birth to the oviparous females; in this manner, over long periods of time the heteroecious type of cycle would become established. As further assistance to the evolutionary process he envisaged an advantage to the migrating species through their having better conditions for multiplication on herbaceous plants in summer than on their original woody hosts on which a phase of depression could often be observed in species which did not emigrate. Mordvilko thus originated the idea of a complementary growth pattern, between the original or primary host and the newer or secondary host, providing nutritional advantages to heteroecious species.

Mordvilko's thinking was limited by the fact that he looked upon heteroecy as an evolutionary end point for aphids rather than as a labile way of life related to prevailing vegetational conditions. This caused him to give rather elaborate arguments to explain the coexistence in the same genera of aphid species living heteroeciously and of others living, without heteroecy, on the plants used only as secondary hosts by the heteroecious species. Hille Ris Lambers (29, 30) explained this phenomenon in much simpler terms as showing that a heteroecy has been lost, the complete cycle now taking place on the original secondary host. Many more examples of this kind of monoecy

are now described than were known to Mordvilko; they demonstrate that there is no finality in heteroecy.

It does appear, however, that apart from heteroecy the evolutionary history of aphids has been marked by rather numerous changes of association from one host to another quite unrelated one. Mordvilko cites a number of such cases, such as the colonization of Aceraceae by *Periphyllus* van der Hoeven (*Chaitophorinella* van der Goot), and of Papilionaceae and Tiliaceae by various Callaphididae (Callipterea). Later, Hille Ris Lambers (30) pointed out that in two plant families only, Rosaceae and Caprifoliaceae, is it now possible to trace a long evolutionary series of aphid genera that suggests parallel evolution of hosts and parasites. He also noted other genera which, while morphologically forming part of the Rosaceous series, now have quite unrelated primary hosts and must have acquired these in relatively recent times, but certainly not by parallel evolution (*Capitophorus* van der Goot, *Cryptomyzus* Oestlund, *Corylobium* Mordvilko). Indeed, it would seem that a large part of the very extensive coverage by aphids of the existing angiosperm floras of temperate zones must be caused by such taxonomically discontinuous host changes rather than to parallel host-aphid evolution.

Inasmuch as such changes are not typical of parasites, the question arises as to how they have been possible for aphids. Shaposhnikov (87) postulated that aphid evolution has followed two main paths, which he called the "specialized" (equivalent to parallel evolution as demonstrated by Hille Ris Lambers) and, alternating with that, the "little specialized" characterized by taxonomically discontinuous host changes. He suggested that all aphid species carry with them the potentiality for the second kind of evolution as well as the first, but that this potentiality is realized only when there occurs a sharp change in the conditions of life on the accustomed host to which the aphids cannot accommodate by changes in themselves. As we see it, the potentiality for host changes resides in the polymorphism of aphids, as was implicit in Mordvilko's theory of the evolution of heteroecy. All aphid species are specialized in two directions, both "vegetatively" as parasites and in their host plants; but within the species there is a division of labour among the forms in this as in other respects. The praesexuales and sexuales have diverse nonassimilatory requirements and responses binding them to particular plants. The more vegetative virginoparae have simpler relations with the host and may therefore be freer to change hosts. They commonly have a wider host range [in *A. fabae* they have been shown experimentally to be less plant-specific and more discriminating plant-physiologically than the gynoparae (51)]. The exception is the first or fundatrix generation of virginoparae hatching from the overwintering egg, which is present when the host reaches its nutritional best at bud-burst and has developed more host-specialization in addition to its vegetative specialization. If, then, the summer viviparae are the potential host truants of the species, as Mordvilko suggested, Shaposhnikov's "catas-

trophic" theory of the conditions required for actual host change seems to us unneccessary.

Field observations and the results of insectary experiment suggest that the raw material of evolutionary host change is produced every season in the shape of vast numbers of alate virginoparae that disperse over the entire plant cover. These alatae are only in degree less vegetatively specialized than the apterous virginoparae, and their offspring, which are occasionally deposited on "wrong" hosts, tend to be apterae. It is true that the production of alatae appears to be associated with present or impending deterioration in nutritional conditions, but the latter is a normal feature of every plant growth cycle and not a rare catastrophe. It is perhaps remarkable, not that aphids have been able to change hosts so often, but that they should nevertheless have retained so much of the more typical phyletic pattern of related genera on related host plants and formed evolutionary series upon them. The ability to survive and reproduce through further generations on a new host is what is rare, not the conditions which test innumerable specimens for such ability.

Three possible paths of evolution have proved open to the rare colonists that succeed. The new host may become a secondary host with regular remigration to the original host, or a complete new cycle may be passed on the new host and lead either to a dual polyphagous cycle or to dichotomy of the original aphid species. Some factors which may affect the path finally followed are suggested below.

Degree of adaptation to the original host.—The longer an aphid lives on a particular host, the closer become the ties binding it thereto. Very long associations may result in specialized gall responses by the host [Hille Ris Lambers (30)], which may become nutritionally indispensable to the generations living in them, especially fundatrices [Zweigelt (106)]. Even in nongalling species the fundatrices are frequently able to mature only on the accustomed primary host or hosts. Mordvilko and Shaposhnikov saw this as a factor favouring the evolution of heteroecy rather than complete transference of the cycle to the new host. This is not the end of the story, for various Aphididae have evolved a complete new cycle on secondary hosts, in which the oviparae and fundatrices resemble the summer generations rather than the original primary host forms, so that the latter have in fact disappeared from the cycle, their functions being discharged by new and less specialized equivalents [Hille Ris Lambers (30)]. Mordvilko was undoubtedly right, however, in maintaining that a period of association of heteroecious type is necessary before such secondary cycles can evolve; and, in fact, such cycles are not known in Pemphigidae, unless the recent primary host association of some Prociphilini (75, 30) is an indication of the existence of a former heteroecy, perhaps from Salicaceae, which was first lost and then replaced by the present heteroecy to roots. Heteroecy, then, has been at least initially promoted by the kind of close bond with the primary host, often involving galling, which prevails in Adelgidae,

Pemphigidae, Thelaxidae, and Aphididae. In Lachnidae, Chaitophoridae, and Callaphididae heteroecy is not known to have evolved at all (unless the Traminae were at one time heteroecious), and here we must suppose that the bond with a particular plant has been of a different kind, perhaps more strictly sensory (it has never resulted in galling, for example, although the hosts are often ancient and the association with them apparently primitive), and that the nature of the bond is perhaps in some way related to the fact that these families have changed hosts only on an all-or-nothing basis.

Growth patterns of the original and new hosts.—Where these are complementary (e.g., woody and herbaceous hosts) the classical theory of Mordvilko and its later developments (49) postulate advantages in the heteroecious way of life. The theory may even apply to heteroecious Fordinae, which over a 2-yr. cycle move from primary to secondary host and back just when heteroecious Aphididae are doing the opposite, even if using the same host genera such as *Rhus* or grasses. For Fordinae exploit the primary host in galls lasting out the summer, and multiply through the ensuing winter on herbs: a manner of extending the reproductive season which Davatchi (13a) suggests can be of special adaptive value in areas with arid summers and moist winters where holocyclic Fordinae are now abundant. But again this is not the whole story. The progression in various aphid genera to secondary monoecy on perennating secondary hosts, already noted, argues that in these cases complementary growth patterns cannot be the dominant factor. Where the growth patterns of the old and new hosts do not seem complementary, but more or less alike, various paths have evidently been open. Even here heteroecy has evolved in some groups (Adelgidae, *Paraprociphilus* Mordvilko, various Hormaphidinae) and may involve a 2-yr. cycle, some times of a highly complex kind [*Hormaphis* Osten-Sacken (28)]. More typical results are either a degree of polyphagy which may or may not extend to overwintering as eggs on unrelated hosts, or an entire dichotomy into separate lineages on the old and new hosts, which give rise ultimately to new taxa.

Dispersal hazards.—Great losses are suffered through failure of migrants to reach suitable hosts, and the repeated dispersals undertaken by heteroecious species are a factor which in changing circumstances could outweigh any gain in productivity from complementary growth patterns in alternate hosts.

Myrmecophily.—Many aphid species are attended by ants (10). Simpler forms of relationship where the aphids are visited but do not receive nest care may favour a given aphid-plant relationship, in that the ants increase the multiplication rate of the aphids and protect them from enemies (5, 10, 20). Increasingly specialized relationships may lead to nest care of the aphids' winter eggs, including regular licking by the ants and consequent increase in percentage hatch (A. J. Pontin, *in litt.*). Obligate myrmecophily may lead to localized distribution of aphid species within the range of dis-

tribution of their host plants [e.g., of 27 known species of aphids in Iceland where ants are absent, all species but one are nonmyrmecophilous elsewhere (31)].

Climatic factors.—Maximum potential for multiplication exists when nutritionally optimum conditions in the host coincide with climatically optimum conditions for the parasites. Herbs tend to continue leaf growth in the temperate summer when mean temperatures are high; whereas the active leaf growth phase on woody plants is passed through in the spring while mean temperatures are low. This again should favour the evolution of ties with herbaceous hosts, irrespective of heteroecy. In moist tropics this effect of climate should tend to disappear under the more uniform conditions. Climate, acting either directly or probably more often through its effects on plant phenology and growth habit, also plays an important role in determining the degree of prevalence of aphid populations maintained by continuous parthenogenesis ("anholocycly"). A discussion of current lines of thought in this field is given by Bodenheimer & Swirski (8). In temperate regions and dry-season tropics or subtropics such cycles are passed most often on herbs (or on woody plants with retreat to their roots in the adverse season), these providing a relatively lenient microclimate and perhaps also some possibility of feeding. In moist tropics many indigenous aphids are able to live anholocyclically on the aerial parts of woody plants.

Those are a few of the more obvious environmental factors shaping the raw material of new host colonizations into the various types of cycle now observed. Their relative effectiveness must vary widely according to the kind of host colonized, and this variability of the whole complex of pressures acting on the aphids forbids any generalizations about the "advantages" of a given way of life. The only generalizations which seem possible from what has been said are:

(*a*) Aphid evolution has combined the normal parasitic trend of close host specificity and parallel evolution, with a rather high frequency of discontinuous host changes. Although such acquisitions of new hosts represent a first step toward polyphagy, they do not seem to have been cumulative within most species because of the strength of opposing pressures favouring host specialization followed by further speciation. As to the nature of the pressures for host specificity, we remain as much in the dark with aphids as with other phytophagous insects. The host changes have provided the opportunities for evolving a very complete coverage of available ecological niches among herbaceous angiosperms, and this is the basis of the success of aphids, and particularly the recent Aphididae, as a group.

(*b*) Aphids have been able to follow this path by their specialized parasitic method of host exploitation, adapted to unstable host plants through polymorphism, and including regular dispersals of great numbers of winged but otherwise parasitic individuals which may be relatively loosely bound to their hosts.

(*c*) Heteroecy has been a frequent result of the breakaway from old hosts. Close host specialization of sexuales or fundatrices and the existence of complementary growth patterns between the old and new hosts may be suggested among factors favouring this result. But it is in no sense an end result and is opposed to the dominant trend to oligophagy.

(*d*) Such extrinsic factors as can be postulated as operating to modify aphid life cycles would now seem in most cases to favour evolution toward monoecy, with or without a bisexual generation, on perennating herbaceous plants. The general trend would not, then, be such as to increase the importance of aphids as pests of annual crops.

(*e*) That, however, is not the only trend. There is a small minority of species typified by *M. persicae* and *A. fabae* which have continued the trend to polyphagy to an extreme. This development may well be recent, and such species gain a notoriety as pests which obscures the fact that they are not typical. But the persistence of their polyphagy and the evolution of such pest species in the future may in some cases be materially facilitated by plant breeding and agricultural practice. The repeated growing of susceptible plants removes previous restraints on evolutionary tendencies to simultaneous polyphagy, to host changing, and to unrestrained host exploitation, and our knowledge of aphid biology is not yet adequate to check them.

LITERATURE CITED

1. Anders, F., *Vitis*, **1**, 142–52 (1957)
2. Anders, F., *Experientia*, **14**, 62–63 (1958)
3. Auclair, J. L., *J. Insect Physiol.*, **2**, 330–37 (1958)
4. Auclair, J. L., *Ann. Rept. Entomol. Soc. Ontario 1957*, **88**, 7–17 (1958)
5. Banks, C. J., *Bull. Entomol. Research*, **49**, 701-14 (1958)
6. Banks, C. J., and Nixon, H. L., *J. Exptl. Biol.*, **35**, 703-11 (1958)
7. Bennett, S. H., *J. Hort. Sci.*, **30**, 252–59 (1955)
8. Bodenheimer, F. S., and Swirski, E., *Aphidoidea of the Middle East* (Weizmann Scientific Press, Jerusalem, Israel, 378 pp., 1957)
9. Bonnemaison, L., *Contribution à l'étude des facteurs provoquant l' apparition des formes ailées et sexuées chez les Aphidinae* (Doctoral Thesis, University of Paris, Paris, France, 1951)
10. Börner, C., and Heinze, K., in Sorauer, P., *Handbuch Pflanzenkrankh.*, 5th ed., **5**(4) (Appel, O., Blunck, H., and Richter, H., Eds., Paul Parey, Berlin and Hamburg, 577 pp., 1957)
11. Broadbent, L., *Biol. Revs. Cambridge Phil. Soc.*, **28**, 350–80 (1953)
12. Chatters, R. M., and Schlehuber, A. M., *Oklahoma Agr. Expt. Sta. Tech. Bull. No. T-40*, 18 pp. (1951)
13. Chibnall, A. C., *Protein Metabolism in the Plant* (Yale University Press, New Haven, Conn., 306 pp., 1939)

13a. Davatchi, A. G., *Rev. pathol. végétale et entomol. agr. France*, **37**, 1–166, (1958)

14. Davey, P. M., *Bull. Entomol. Research*, **45**, 539–51 (1954)
15. Davidson, J., *Ann. Appl. Biol.*, **12**, 472–507 (1925)
16. Day, M. F., and Irzykiewicz, H., *Australian J. Biol. Sci.*, **6**, 98–108 (1953)

16a. Dixon, A. F. G., *Trans. Roy. Entomol. Soc.* (*London*), **110,** 319–34 (1958)
17. Dunn, J. A., *Bull. Entomol. Research,* **40,** 97–122 (1949)
18. Eastop, V. F., *Nature,* **176,** 936 (1955)
19. El-Ziady, S. (In manuscript, 1958)
20. El-Ziady, S., and Kennedy, J. S., *Proc. Roy. Entomol. Soc.* (*London*), **[A]31,** 61–65 (1956)
21. Evans, A. C., *Trans. Roy. Entomol. Soc.* (*London*), **89,** 13–22 (1939)
22. Evenhuis, H. H., *Ecological Investigations on the Woolly Aphid,* Eriosoma lanigerum (*Hausm.*) *and its parasite* Aphelinus mali (*Hald.*) *in the Netherlands* (Doctoral Thesis, University of Groningen, Haren, Groningen, Netherlands, 1958)
23. Haine, E., *J. Animal Ecol.,* **24,** 388–94 (1955)
24. Haine, E., *Nature,* **175,** 474 (1955)
25. Heathcote, G. D., *Ann. Appl. Biol.,* **45,** 133–39 (1957)
26. Hijner, J. A., and Cordon, F. M., *Mededel. Inst. Rationele Suikerprod.,* **23,** 251–70 (1953)
27. Hill, A. R., *Bull. Entomol. Research,* **48,** 467–76 (1957)
28. Hille Ris Lambers, D., *In het Voetspoor van Thijsse* (*Wageningen*), 328–43 (1949)
29. Hille Ris Lambers, D., *Temminckia* (*Leiden*), **8,** 182–324 (1949)
30. Hille Ris Lambers, D., *Proc. Intern. Congr. Entomol., 8th Meeting* (*Stockholm, Sweden, 1948*), 141–44 (1950)
31. Hille Ris Lambers, D., in *Zoology of Iceland,* **3**(52a), 29 pp. (Ejnar Munksgaard, Copenhagen and Reykjavik, 1955)
32. Hiratsuka, E., *Bull. Imp. Sericult. Expt. Sta. Japan,* **1,** 137–315 (1920)
33. Ito, Y., *Researches Population Ecol.* (*Kyoto*), **1,** 36–48 (1952)
34. Ito, Y., *Nôgyô Gijutsu Kenkyûjo Hôkou,* [C]**4,** 187–99 (1954)
35. Johnson, B., *Proc. Conf. Potato Virus Diseases, 2nd Meeting* (*Lisse-Wageningen, Netherlands, 1954*), 70–74 (1955)
36. Johnson, B., *Australian J. Sci.,* **18,** 199–200 (1956)
37. Johnson, B., *J. Insect Physiol.,* **1,** 248–56 (1957)
38. Johnson, B., *J. Linnean Soc. N. S. Wales,* **82,** 191–98 (1957)
39. Johnson, B., *Nature,* **181,** 205 (1958)
40. Johnson, B., *Animal Behaviour,* **6,** 9–26 (1958)
41. Johnson, C. G., *Biol. Revs. Cambridge Phil. Soc.,* **29,** 87–118 (1954)
42. Johnson, C. G., *Rothamsted Exptl. Sta. Rept. 1955,* 191–201 (1956)
43. Johnson, C. G., *J. Animal Ecol.,* **26,** 477–92 (1957)
44. Johnson, C. G., Taylor, L. R., and Haine, E., *Ann. Appl. Biol.,* **45,** 682–701 (1957)
45. Karafiat, H., and Franz, J., *Zool. Jahrb.* (*Systematik*), **84,** 467–504 (1956)
46. Kennedy, J. S., *Proc. Intern. Congr. Entomol., 8th Meeting* (*Stockholm, Sweden, 1948*), 423–26 (1950)
47. Kennedy, J. S., *Nature,* **168,** 890–94 (1951)
48. Kennedy, J. S., *Biol. Revs. Cambridge Phil. Soc.,* **31,** 349–70 (1956)
49. Kennedy, J. S., *Entomol. Exptl. et Appl.,* **1,** 50–65 (1958)
50. Kennedy, J. S., *Proc. Intern. Congr. Entomol., 10th Meeting* (*Montreal, Que., Canada, 1956*) **2,** 397–404 (1958)
51. Kennedy, J. S., and Booth, C. O., *Ann. Appl. Biol.,* **41,** 88–106 (1954)
52. Koch, A., *Verhandl. deut. zool. Ges.* (*Erlangen*), 328–48 (1955)

53. Lal, R., *Indian J. Entomol.*, **17**, 52–62 (1955)
54. Lamb, K. P., *J. Insect Physiol.*, **3** (In press)
55. Lees, A. D., *Proc. Symposium Photoperiodism and Related Phenomena Plants and Animals (Gatlinburg, Tenn., November, 1957)* (In press, 1958)
56. Lees, A. D., *J. Insect Physiol.*, **3** (1959) (In press)
57. Legay, J. M., *La prise de nourriture chez le Vers à Soie* (Doctoral Thesis, University of Paris, Paris, France, 1957)
58. Lindemann, C., *Z. vergleich. Physiol.*, **31**, 112–33 (1948)
59. Lipke, H., and Fraenkel, G., *Ann. Rev. Entomol.*, **1**, 17–44 (1956)
60. Maltais, J. B., *Can. Entomologist*, **84**, 291–94 (1952)
61. Marcovitch, S., *J. Econ. Entomol.*, **28**, 62–70 (1935)
62. Markkula, M., *Ann. Zool. Soc. Zool. Botan. Fennicae Vanamo*, **15**(5), 113 pp. (1953)
63. McAllan, J. W., and Cameron, M. L., *Can. J. Zool.*, **34**, 559–64 (1956)
64. Milne, A., *Can. Entomologist*, **89**, 193–213 (1957)
65. Mittler, T. E., *The Feeding and Nutrition of Aphids* (Doctoral Thesis, University of Cambridge, Cambridge, England, 1954)
66. Mittler, T. E., *J. Exptl. Biol.*, **34**, 334–41 (1957)
67. Mittler, T. E., *J. Exptl. Biol.*, **35**, 74–84 (1958)
68. Mittler, T. E., *J. Exptl. Biol.*, **35**, 626–38 (1958)
69. Mittler, T. E., *Proc. Roy. Entomol. Soc. (London)*, [A]**33**, 49–55 (1958)
70. Moericke, V., *Z. Tierpsychol.*, **7**, 265–74 (1950)
71. Moericke, V., *Z. angew. Entomol.*, **37**, 29–91 (1955)
72. Moericke, V., *Z. Pflanzenkrankh. u. Pflanzenschutz*, **62**, 588–93 (1955)
73. Moericke, V., *Proc. Conf. Potato Virus Diseases, 2nd Meeting (Lisse-Wageningen, Netherlands, 1954)*, 55–69 (1955)
74. Moericke, V., *Z. Pflanzenkrankh. u. Pflanzenschutz*, **64**, 507–14 (1957)
75. Mordvilko, A. K., *Ann. Mag. Nat. Hist.*, [10]**2**, 570–82 (1928)
76. Müller, H. J., *Beitr. Entomol.*, **3**, 229–58 (1953)
77. Müller, H. J., *Z. angew. Entomol.*, **38**, 82–96 (1955)
78. Müller, H. J., *Sitzber. deut. Akad. Landwiss. Berlin*, **5**(27), 20 pp. (1956)
79. Müller, H. J., *Entomol. Exptl. et Appl.*, **1**, 66–72 (1958)
80. Nielson, M. W., and Barnes, O. L., *J. Econ. Entomol.*, **50**, 805–7 (1957)
81. Nuorteva, P., *Entomol. Exptl. et Appl.*, **1**, 41–49 (1958)
82. Painter, R. H., *Ann. Rev. Entomol.*, **3**, 267–90 (1958)
83. Painter, R. H., *Proc. Intern. Congr. Entomol., 10th Meeting (Montreal, Que., Canada, 1956)* **3**, 451–58 (1958)
84. Pathak, M. D., and Painter, R. H., *Ann. Entomol. Soc. Am.*, **51**, 250–54 (1958)
85. Peters, D. C., and Painter, R. H., *Kansas Agr. Expt. Sta. Tech. Bull. No. 93*, 44 pp. (1958)
86. Richards, A. G., and Brooks, M. A., *Ann. Rev. Entomol.*, **3**, 37–54 (1958)
87. Shaposhnikov, G. Ch., *Shteniya pamyati Nikolaya Alexandrovich Cholodkovskovo 1950*, 28–60 (*Izdatelstvo Akad. Nauk. S.S.S.R.*, 1951)
88. Smith, B. D., *A Study of the Factors Affecting the Populations of Aphids on on* Sarothamnus scoparius (*L.*) (Doctoral Thesis, University of London, London, England, 1957)
89. Smith, L. M., *J. Agr. Research*, **54**, 345–64 (1937)
90. Sokolov, A. M., and Sokolova, R. A., *Doklady Vsesoyuz. Akad. Sel'skokhoz. Nauk im. V. I. Lenina*, **17**(2), 12–18 (1952); *Rev. Appl. Entomol.*, [A]**41**, 257 (1953)

91. Tambs-Lyche, H., and Kennedy, J. S., *Entomol. Exptl. et Appl.*, **1**, 225–39 (1958)
92. Taylor, L. R., *Proc. Linnean Soc. London*, **169**, 67–72 (1958)
93. Treherne, J. E., *J. Exptl. Biol.*, **35**, 297–306 (1958)
94. Unger, K., and Müller, H. J., *Züchter*, **24**, 338–45 (1954)
95. Van Hoof, H. A., *An Investigation of the Biological Transmission of a Non-Persistent Virus* (Doctoral Thesis, University of Wageningen, Wageningen, Netherlands, 1958)
96. Van Soest, W., and de Meester-Manger Cats, V., *Virology*, **2**, 411–14 (1956)
97. Waterhouse, D. F., *Ann. Rev. Entomol.*, **2**, 1–18 (1957)
98. Watson, M. A., and Nixon, H. L., *Ann. Appl. Biol.*, **40**, 537–45 (1953)
99. Way, M. J., *Plant Pathology*, **7**, 9–13 (1958)
100. Way, M. J., and Banks, C. J., *Proc. Intern. Congr. Entomol., 10th Meeting (Montreal, Que., Canada, 1956)* **4**, 907–9 (1958)
101. Weatherley, P. E., Peel, A., and Hill, G. P., *J. Exptl. Bot.*, **10**, 1–16 (1959)
102. Wellington, W. G., *Can. J. Zool.*, **35**, 293–323 (1957)
103. Wertheim, G., *Trans. Roy. Entomol. Soc. (London)*, **105**, 79–96 (1954)
104. Wigglesworth, V. B., *The Principles of Insect Physiology*, 5th ed., (Methuen & Co., London, England, 546 pp., 1953)
105. Wigglesworth, V. B., *The Physiology of Insect Metamorphosis* (University Press, Cambridge, England, 152 pp., 1954)
106. Zweigelt, F., *Monograph. angew. Entomol.*, **11** (*Z. angew. Entomol.*, **17**, 684 pp., 1931)

THE BIOLOGY OF PARASITIC HYMENOPTERA[1]

By Richard L. Doutt
Department of Biological Control, University of California, Berkeley, California

This review restricts its coverage to those parasitic Hymenoptera that are sometimes designated as "parasitoids." Such a limitation on coverage is not particularly confining, however, for the parasitic Hymenoptera number in the thousands of species, occur throughout the world, possess complex and fascinating biologies, and frequently function as determinants of host population densities. Because of the last attribute, the parasitoids are of tremendous economic importance and are therefore commonly employed as biological control agents.

It has been noted that these particular entomophagous Hymenoptera differ from true parasites in ways sufficient to set them apart and, accordingly, to justify the use of the distinguishing term "parasitoid." They are recognized as being different because: (*a*) the development of an individual destroys its host; (*b*) the host is usually of the same taxonomic class, *i.e.*, Insecta; (*c*) in comparison with their hosts, they are of relatively large size; (*d*) they are parasitic as larvae only, the adults being free-living forms; (*e*) they do not exhibit heteroecism; (*f*) as a parameter in population dynamics their action resembles that of predators more than that of true parasites.

Although there may be justification for using the term "parasitoid," it does not seem to have gained widespread usage, and since it has been adopted by few authorities in this field, it is in this review used interchangeably with "parasite."

Parasites are readily distinguished from predators, and the test here is whether in their development they consume merely a single host individual or must devour several in order to reach maturity. While parasites for their development require only a single host individual and are distinguished on this basis, they are subject to further classification into many subcategories depending on the mode of attack and the type of host. Accordingly, if the parasite develops within the host's body it is an internal or endoparasite; whereas if it feeds from an external position, it is called an external or ectoparasite. A parasite is termed solitary if only one individual develops per host, but many species habitually develop several progeny on a single host and are therefore said to be gregarious. These simple categories are often combined so that there are solitary internal parasites as well as solitary external ones, and, of course, the gregarious species, too, may be either internal or external feeders.

Classification does not stop here. Since all stages of insect hosts are subject to attack, we find that some species are egg parasites, other are larval

[1] The survey of literature pertaining to this subject was completed in January, 1958.

parasites, some attack pupae, and a few parasitize adults. There may be intermediate categories, such as are seen in certain braconid genera like *Chelonus* or *Ascogaster,* in which the host egg stage is attacked, and yet the parasite continues to develop within the host larva. These are appropriately termed egg-larval parasites, and Clausen (19) has recently summarized the Hymenoptera with this habit. Any serious student of parasitic groups should also consult Clausen's book on entomophagous insects (16).

There is a widely published principle, although by no means proved, that the more complete the tolerance of a host species to its parasitic fauna, the longer the relationship must have existed. Adherents to this view would therefore consider that the parasitoid which kills its host is a comparatively unadapted type, and is like a predator in reflecting a primitive condition. This view is well illustrated in the following passage from Wardle & Buckle (80):

> The more completely adapted the parasite be to its host, the longer will be its connection with it, and the less its liability to injure it. Tolerance of a parasite by a host organism, therefore, would seem to imply an interrelationship of long standing; an organism that kills its host is, comparatively speaking, a newcomer to the ranks of parasitism; it has not yet attained the status of a true parasite; it is just as much a predator as is the organism which lives by violently assaulting and immediately devouring weaker forms of life.

On the other hand, Ball (5) refuses to accept a high degree of pathogenicity as prima-facie evidence of recent development of the host-parasite relationship. He thinks there are instances in which the parasite, as it became better adapted to life in its host, became more, rather than less, capable of producing disease. "Even a parasite may find aggressiveness more attractive and more valuable than an existence of peace and symbiosis."

By extending either of these views one can quickly become involved in the area of population dynamics and the role of the exploitation of hosts by parasitoids in the evolution of the ecosystem. This, however, is beyond the scope of this paper; a treatment of the subject is to be found in Allee *et al.* (1).

It has been said by Allee *et al.* (1) that living organisms, as hosts to parasites, form one of the three major habitats on earth, comparable to the aquatic and terrestrial habitats in which the hosts themselves dwell. Therefore, with each host as a kind of physiological island, it is not surprising that speciation has apparently occurred frequently among the parasitoids. It is not difficult to see how extrinsic factors associated with the host might set up isolating mechanisms in parasite populations attacking it. If these were sufficient to interrupt gene flow between portions of the parasitoid species and lasted for any substantial period of time, then perhaps a new species would evolve. In this connection it may be profitable to examine the ways in which potential hosts and parasitoids may be brought together in nature to establish a host-parasite relationship. Also it may be well to exa-

mine those forces that are disruptive or deterrent to the proper functioning of a host-parasite relationship.

It seems obvious that to achieve a host-parasite relationship the two species must first be seasonally, geographically, and ecologically coincident. But even when these requirements are met, the parasitic relationship may still not be established if there are physical, psychological, physiological, or nutritional barriers.

Diapause

One of the phenomena which seems to operate to overcome the temporal barrier and thereby to harmonize the cycles of host and parasite is diapause. This is the well-known physiological state of arrested growth or reproduction that occurs in insects. The intervention of diapause in some stage of the life cycle of a parasitic species is often essential if there is to be synchronization of development between host and parasite. This is particularly true if the host is univoltine. Flanders (33) has considered diapause in the parasitic Hymenoptera to be adaptive in that it delays development until the host attains the stage presumably most suitable for the nutritional requirements of the parasite. Simmonds (66, 67, 68), on the other hand, believes that the coincidence of diapause with unfavorable environmental conditions is usually fortuitous, and he concludes that fundamentally ". . . diapause is due to some physiological maladjustment during development, and is not an adaptive character enabling a species to survive a period unfavorable to further growth, but a pathological state due to previous environmental or intrinsic influences."

The causes and the manifestations of diapause in the parasitic Hymenoptera are not simple, and generalizations are usually rendered meaningless by many exceptions. As Andrewartha (4) has indicated, the phenomenon of diapause is not always clear-cut and easily recognizable, and furthermore, with the parasitic Hymenoptera, it is particularly difficult to unravel the causal relationship between the parasite's environment and the inception of diapause. In many species, as both Flanders (33) and Simmonds (68) have noted, the individuals may enter a state of diapause at a time when the environment is favorable to continuous development and increase of the species.

Among the progeny of a single female there may be some individuals that undergo diapause and others that do not. Simmonds (66, 67, 68) found that the physiological state of the parent female prior to and at the time of oviposition influenced the proportion of her progeny that entered diapause. In the case of the pteromalid *Spalangia drosophilae* Ashmead, it was shown (66) that as the female gets older an increasing percentage of her progeny enters diapause. In the case of an ichneumonid, *Cryptus inornatus* Pratt, it was apparent (67) that progeny from those females which in development had passed through a period of diapause show a far lower incidence of diapause than do progeny from females which developed without a diapause.

Furthermore, Simmonds (68) found that changing an adult female *Cryptus* from a diet of cane sugar to a diet of cane sugar and raisin not only increased longevity and egg production but also raised the incidence of diapause in the progeny from 2.5 per cent to 36.5 per cent. In addition to the influence of maternal physiology on the incidence of diapause, these studies also showed that the incidence (in *Spalangia*) increased if low temperatures prevailed during development, and the incidence was raised (in *Cryptus*) if the quality of the larval food was changed from *Loxostege* to the unnatural host *Pyrausta*.

The recent work of Schneiderman (64) gives support to Simmond's findings on the influence of maternal physiology on diapause in the progeny. Schneiderman believes that the triggering stimulus acts at an early stage in the life cycle while actual diapause is not manifested until much later. He shows that in *Nasonia* (= *Mormoniella*) exposure of the female wasps to low temperatures during ovigenesis causes diapause in the offspring at the close of the last larval instar. Schneiderman found further that temperatures below 15°C. were necessary to break diapause in larval *Nasonia*, but that prolonged anaerobiosis prevented chilling from breaking diapause.

It is Schneiderman's (64) hypothesis that inhibition of neurosecretory cells causes diapause and that, in insects with facultative diapause, inactivation of the brain may be triggered by many things. It seems well established that in some endoparasitic species diapause is induced only by being in hosts that are themselves in a condition of diapause. A splendid example of this is shown by *Trichogramma cacoeciae* Marchal when it parasitizes the eggs of *Archips rosana* Linnaeus. The parasite is in diapause in the host from July until March, when it emerges and parasitizes the remaining eggs of the same host egg mass which are then no longer in diapause. This second generation of *T. cacoeciae* in the postdiapause eggs of *A. rosana* does not enter diapause.

Lees (49) recognizes the assumption that some diapause substance has been ingested by *T. cacoeciae* and that this in turn inhibits the growth of the parasite, but he offers a different and equally consistent explanation for the life cycle of *T. cacoeciae* on *A. rosana*. The same result would occur, he asserts, if some general seasonal factor, such as length of day, acted differentially upon the parasite in spring and summer. There is support for Lees' suggestion, and he cites the work of Gayspitz & Kyao (44), who found that in the relationship of *Apanteles glomeratus* (Linnaeus) and *Pieris brassicae* (Linnaeus) the host and parasite respond quite independently to the external environment. In both of these insects the photoperiod is important and in both diapause is prevented during long days and induced during short days. There is, however, a slight disparity in the critical photoperiod sufficient to set them apart. Thus, although *A. glomeratus* is responsive to the length of day while still endoparasitic, it remains sensitive for some time after leaving the host. The parasite can therefore be prevented from entering diapause by exposure to a long photoperiod immediately after emergence from the host.

According to Lees (49), "Synchronous development is most striking when the host possesses a facultative diapause or when the parasite can develop in two species that differ in voltinism." *Chelonus annulipes* Wesmael remains in the first instar until its host, *Pyrausta,* spins its cocoon. Accordingly, the first instar may be greatly prolonged if the host undergoes diapause. *C. annulipes* completes its life cycle in less than two months in the first (nondiapause) generation of the multivoltine strain of *Pyrausta;* it requires about 10 months in the second (diapause) generation of this strain; whereas in the univoltine strain of *Pyrausta* 12 months are required.

Because an insect in diapause does not respond immediately to any ordinary amelioration of the environment and since the resting state in *C. annulipes* is terminated by a direct response to physiological changes in the host, it is suggested by Andrewartha (4) that *Chelonus* is not in diapause. He inquires, however, whether the *Chelonus* first instar larva would be competent in an adequate environment to resume development without any prolonged resting stage, and he suggests that this question might be answered experimentally by transplanting parasite larvae from early stage *Pyrausta* larvae to older hosts near pupation. Lees (49, 50) calls attention to a transplantation experiment done by Schneider (62, 63), who was studying the restraint exercised by syrphid hosts on the ichneumonid *Diplazon fissorious* Gravenhorst. Schneider showed that if young larvae, activated by the pupation of their host, *Epistrophe balteata* Degeer, are put into diapausing larvae of *Epistrophe bifasciata* Fabricius they are immediately immobilized. Conversely, dormant larvae are instantly activated when they are transplanted to a pupating *E. balteata.* In future studies of this type, workers may find it profitable to adopt the technique of Bronskill & House (8), who have been able to rear to maturity on unnatural food the transplanted larvae of the endoparasitic ichneumonid *Pimpla turionellae* (Linnaeus).

In the parasitic Hymenoptera there are records of diapause occurring in every stage of development. Flanders (28, 33) states that the undeposited eggs of many species are in a state of diapause as long as they are stored in oviducts or modified portions of the ovary, since further development is dependent on immersion in the nutrient body fluids of the host. However, this may be quiescence rather than diapause. In the larval and pupal stages diapause is not uncommon, and there are some interesting records of imaginal diapause. Flanders (29, 33) found that the black scale parasite, *Metaphycus helvolus* Compere, is forced into an imaginal diapause if it is isolated from its host for two or three weeks. Ovigenesis ceases and the undeposited eggs are absorbed. The cessation of the diapause is brought about only by the feeding of the female on the body fluids of the host. Andrewartha (4) agrees that diapause in the adult stage may take the form of a failure to ripen eggs or sperms and may be manifest by an extended preoviposition period. As an example of this he cites the work of Skoblo (69) on *Habrobracon brevicornis* (Wesmael). The preoviposition period of the adult was greatly prolonged by subjecting the feeding larvae to temperatures in the lower ranges.

Incidental to this general subject of diapause in the parasitic Hymenoptera, and somewhat on the other side of the coin, are observations that the period of diapause in host insects may be shortened by parasitization. Parker (56) showed that the larvae of the satin moth parasitized by *Apanteles solitarius* (Ratzeburg) terminate their diapause earlier than do unparasitized larvae. A similar observation was made by Varley & Butler (78) on parasitized larvae of a chloropid. The recent work of Schneider (62, 63) showed that *Diplazon pectoratorius* (Thunberg) caused premature pupation in its syrphid host. His ingenious experiments showed that the induced pupation is the result of the direct action of some substance secreted by the parasite.

Host Specificity

An analysis of host specificity in the parasitic Hymenoptera discloses many of the factors that determine whether or not any two given species are to be associated as host and parasitoid. While there are a few species that limit their attack to a single host species, most parasitoids will be found in nature to attack several different hosts. None of these parasites appears to be completely indiscriminate, however, and under natural conditions a parasite will attack only a fraction of the species on which development is actually possible. In the artificial conditions of a laboratory one can remove the barriers of time and space which separate potential hosts and parasites in nature. The fact that parasites may be bred in the laboratory on unnatural hosts is often of great importance in the mass culture of parasites for field colonizations. Certain insects are more amenable to insectary production than others, and when these can be used as factitious hosts the mass culture of the parasitic species is simplified and can often be put on a commercial basis. Recent examples of this are the use of the potato tuberworm, *Gnorimoschema operculella* (Zeller), in the production of the oriental fruit moth parasite, *Macrocentrus ancylivorus* Rohwer [Flanders (31); Finney *et al.* (26)] and the breeding of *Aphytis maculicornis* Masi for the control of *Parlatoria oleae* (Colveé) on the factitious host, *Hemiberlesia lataniae* (Signoret).

Although *M. ancylivorus* and *A. maculicornis* readily accepted unnatural hosts in the laboratory, this was not the case with *Horogenes molestae* (Uchida). By selective breeding, Allen (2) overcame the reluctance of this parasite to attack the potato tuberworm. In 1941, Salt (60) wrote that "an instance in which the parasite actually chooses its own host species in preference to the ancestral host has yet to be described among entomophagous forms." Perhaps Allen's paper is an indication that a change of preference may someday actually be described.

The obvious fact that a parasite limits its attack to a fraction of the suitable host species that occur in nature has stimulated investigations of the behavior of the adult parasites and the criteria used by the females in

selecting their hosts. From this work it appears that four distinct and consecutive processes of selection occur [Salt (59); Flanders (41)], and through their operation the natural host list of a parasite becomes restricted. The four steps are: (*a*) host habitat finding, (*b*) host finding, (*c*) host acceptance, and (*d*) host suitability.

Host habitat finding.—The first factor which eliminates many species from the potential host list of a parasite is the failure of the habitats of the two species to coincide. A parasite initially and fundamentally seeks a certain environment, and it does this irrespective of the presence or absence of hosts. Laing (48) showed that some parasites, when ready to oviposit, do not immediately seek the host itself but first search for a special kind of situation. Thus *Alysia manducator* Panzer is a parasite of maggots which live in decomposing carcasses, but it is attracted to meat whether or not the latter contains, or has ever contained, host larvae. Similarly, *Nasonia* (= *Mormoniella*) *vitripennis* (Walker) is also attracted to meat even though no hosts are present. It is a parasite of fly pupae, however, and its attraction to old meat where pupae might be present is more pronounced than to fresh meat. Thorpe & Jones (74) showed *Idechthis* (*Nemeritis*) *canescens* (Gravenhorst) is attracted by smell of oatmeal, even though its host, *Ephestia,* is not present. *Stomatoceras rubrum* Ashmead, parasitic on the ant lion, *Myrmeleon,* is attracted to sand, and Wallace (79) found that it evinces interest in any kind of small depression.

There is evidence that shows clearly the attraction a particular plant species may have for the parasite which may or may not find hosts there. Smith (70) reports that two species of *Aphytis* liberated in a small greenhouse showed marked difference in preferences for California red scale, *Aonidiella aurantii* (Maskell), growing on different plant species. Smith reared a ratio of *Aphytis chrysomphali* (Mercet) to *Aphytis lingnanensis* Compere of 1:3 from hosts growing on *Yucca,* whereas on sago palm the ratio was 1:81. Other examples of the effect of the plant composition on the work of the parasites have also been reported. The ichneumon *Collyria calcitrator* Gravenhorst controls its host, *Cephus pygmaeus* Linnaeus, more effectively in wheat fields than in barley fields. *Hyposoter disparis* Viereck prefers dense woodlands and seldom attacks hosts that inhabit the edges of clearings.

The distribution of parasites is influenced also by the concentration of food sources for the adults. Allen & Smith (3), studying *Apanteles medicaginis* Muesebeck, found that certain areas contain many sources of food for adult *Apanteles* and thus favor increased longevity and fecundity. They found that in these areas there is generally a higher degree of parasitism of the host, *Colias eurytheme* Boisduval, than in localities where there is not an abundant source of plant nectaries and aphid honeydew. Similarly, Clausen *et al.* (20) found that *Tiphia matura* Allen and Jaynes lacks effectiveness because it is limited by its adult food habits to areas smaller than those occupied by its host, the Japanese beetle. The hosts of an eucharid parasite may be entirely free from attack over most of their range because of the

limited distribution of the plants which stimulate egg deposition by the parasite. [Clausen (17)].

Lloyd (51), in making observations on some thynnids in western Patagonia, noticed that two species occurred in the same sections of pasture and forest for several seasons and maintained themselves as more or less independent units. By marking females, evidence was obtained which suggested that at the conclusion of the mating flight the winged males tended to return the apterous females to the sites where they picked them up. Consequently Lloyd questions the views of earlier workers who believed that the mating flight served primarily in the dissemination of the species.

Host and parasite populations will exhibit differential distribution of their progeny if the adults respond differentially to a single environmental factor. This has been shown by Burnett (10, 11), who found that there was a difference in the modal temperature of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), and its parasite, *Encarsia formosa* Gahan. In his later studies (12, 13, 14) on the eulophid *Dahlbominus fuscipennis* (Zetterstedt), Burnett found that the number of hosts (*Neodiprion*) parasitized, the number of parasite eggs laid, and the rate of change in parasitism were greatly modified by change in temperature. Although this differential dispersion of the host and parasite would seem to be a disruptive factor in the functional relationship of the two species, Burnett (11) believes the host and parasite show mutual adaptations for the preservation of the system and (10) that the differential dispersion appears to be essential for the survival of both species under certain conditions.

Host finding.—Once the parasite is in the habitat of the host, it still must be able to locate a host individual. There have been a number of studies on the biology of searching, most of which have been prompted by the desire to determine whether searching is random or directed. It would appear that most of the results indicate there is something of a compromise between random and nonrandom searching. For example, the searching of the natural environment by *Angitia* sp., a parasite of *Plutella maculipennis* Curtis, has been shown to give results midway between what would be expected from a random search and what would accrue from a systematic one [Ullyett (75, 76)].

Of the senses used by the parasite in detecting the presence of hosts, the one which has most commonly been reported is the sense of smell. Ullyett (77) witnessed the remarkable swarming habits of the ichneumonid *Pimpla bicolor* Bouche, a pupal parasite of the moth *Euproctis terminalis* Walker, on pines in South Africa. If a cocoon of the moth is broken open in the forest, both the pupa from the cocoon and the hands and arms of the observer are covered by a swarm of the parasite females within the matter of a few minutes, although few or no parasites may have been observed in the vicinity previously. The normal attraction of the pupa within the cocoon is no doubt intensified by breaking open the latter.

Ullyett (77) notes that a parasite tends to search in the parts of its en-

vironment most likely to contain its host and that this is caused by a combination of preadaptation in habits and a specific attraction exerted by the portion of the environment concerned. He repeatedly noticed in releasing *Chelonus texanus* Cresson against the Karoo caterpillar, *Loxostege frustralis* Walker, that no matter how high up the adults were released, they always immediately dropped to the level of the low Pentzia bushes on which the host occurred. Only then did they commence their search or dispersal over the area. The Pentzia bushes are approximately the same height as the beet crops on which the original host of the parasite, *Loxostege sticticalis* (Linnaeus), occurs. The parasite is therefore preadapted to search at a particular height above ground level.

Host acceptance.—Even though a parasite actually finds or contacts a suitable host, it still may not attack it if the proper stimuli are lacking. This acceptance of the host by the parasite is the third step in the process of determining the host specificity of the parasite. This step is truly host selection.

Requisites for attack may be such things as host odor, location, size, shape, or even motion. Smith (71) found that *Microctonus vittatae* Muesebeck is stimulated to oviposition principally by the motion of its host, *Phyllotreta*. Motionless beetles were palpitated by the wasp's antennae and caused to move before the wasps would attack. Bryden & Bishop (9) reported the same phenomenon in a study of *Perilitus coccinellae* Schrank.

This matter of host acceptance or host selection is clearly a matter of behavior and, according to Salt (59), might be distinguished as the "psychological selection of hosts." My colleague, Dr. C. B. Huffaker, suggests that "ethological selection" is a better term.

Flanders (27, 32) believes that the act of mating or the presence of sperms in the spermatheca has a marked effect on the psychology of the female. In certain Aphelinidae, mating has a remarkable psychological effect in that it causes significant change in the type of host selected and the manner of oviposition. Extreme examples occur in the genera *Aneristus, Casca, Coccophagus, Euxanthellus,* and *Physcus.* When unmated, the females of certain species in these genera oviposit only hyperparasitically in an insect already parasitized by the same or similar species. Consequently, the male develops only as a primary parasite of the immature instars of its own or similar species, and the host of the male is never the host of the female, nor the host of the female the host of the male.

It is well known that many parasites have the ability to discriminate between parasitized and healthy hosts and thus avoid superparasitism. Flanders (37) pointed out that this differentiation may result from an odor left on the host by the parasite which first contacts it. This Flanders terms the "spoor effect." It has been suggested by Simmonds (65) that there are chemoreceptors on the ovipositor of *I. canescens.* This was demonstrated by Dethier (22), who, in using a technique similar to that employed in testing tarsal chemoreceptors, showed that *I. canescens* responds to chemical stimu-

lation of the exsheathed ovipositor. Dethier suggests:

> Either the sensilla which are located on the shaft of each valvula subserve a chemoreceptive function, or the stimulating solutions diffuse through the general cuticle of the organ, or the solutions are advanced by capillarity up the egg tube formed by the appressed surfaces of the valvulae to the region of the genital openings where they may act upon sensitive areas.

Apparently it is through similar sense organs that *Stenobracon deesae* (Cameron) can distinguish healthy from parasitized hosts. This parasite attacks *Chilo* larvae which are stem borers in graminaceous crops. According to Narayanan & Chaudhuri (55), the only possible way *S. deesae* can judge the quality of the host seems to be by means of its ovipositor:

> It is probable that when a female *Stenobracon* inserts its ovipositor into a host to paralyze it before oviposition, she receives a stimulus from a healthy host which is different from that derived from a parasitized host.

Host suitability.—Even though a hymenopterous parasite has found the potential host in its habitat and selected it for attack, the host-parasite relationship may still not succeed if the potential host individual is immune or otherwise unsuitable. This matter of host suitability is, then, the fourth and final step in the process by which the host list of a parasite species becomes restricted. Bess (7) has recognized this and accordingly has said that oviposition by a parasite is not necessarily an index to host suitability, the attractiveness of the host being often independent of its suitability for parasitic development.

The suggestion that a once susceptible host population may become resistant to parasitic attack is to be found in the work of Muldrew (54). In Canada the larch sawfly, *Pristiphora erichsonii* (Hartig), was for years heavily parasitized by *Mesoleius tenthredinis* Morley, but it later became apparent that in Manitoba and Saskatchewan the efficiency of the parasite had decreased greatly. At the same time, however, the parasite continued to be very effective in British Columbia. Muldrew's (54) studies showed that the hosts in Manitoba and Saskatchewan were inhibiting the parasite's embryonic development, and that this was correlated with the deposition of phagocytic capsules around the embryos. He did not find the phenomenon occurring in the British Columbia stocks even though the sawflies would encapsulate foreign bodies such as rose thorns. When Muldrew removed encapsulated and inhibited larvae, he found that they would hatch in Ringer's solution. This work offers one of the clearest examples of phagocytes' playing an important role in the immunity reaction of a host.

For some time it has been recognized that the host plant may confer on the host insect a kind of immunity to parasitization [Flanders (41); Smith (70)]. Recently Smith (70) has shown that while *Habrolepis rouxi* Compere suffers very little mortality (3.1 per cent) of its immature stages when attacking California red scale, *Aonidiella aurantii* (Maskell), on grapefruit, when the red scale is grown on sago palm 100 per cent mortality of im-

mature *H. rouxi* occurs. He found a similar host plant induced immunity in the red scale to another internal parasite, *Comperiella bifasciata* Howard.

Effect of hosts on their parasitoids.—Closely related to the matter of host suitability is the effect of hosts on their insect parasites. Salt (60) has written:

> Far from being a purely passive victim, obliterated without a trace, the host is often able to impress its mark, and a very clear mark at that, upon the insect parasitoid that destroys it. Proof is already available of effects of the host on the size, form, rate of development, and behaviour of its parasite; evidence can be found of influence on fecundity, longevity and vigour; and it is likely that many other effects still await discovery and description. There can be no doubt that the host may bequeath to its parasite an important and sometimes striking legacy of morphological, physiological, and behaviouristic characters.
>
> It follows that the host is one of the important factors that must be considered and controlled in any critical, and especially any quantitative, study of insect parasitoids. Accounts of behaviour, data on rate of reproduction and longevity, measurements of length, even descriptions of structure—none of these have any absolute validity for the species, none of them can be considered "biological constants" of a parasitoid, unless the host is known and recorded.

Salt (60) concluded that perhaps the most generally applicable result of his paper was

> . . . the demonstration of the need in insect parasitology to rear parasites from known and standardized hosts, as well as under controlled laboratory environmental conditions, if data of general value are to be obtained.

The effect of the size of the host on the sex ratio of its hymenopterous parasites is summarized by Clausen (15). He cites a number of examples in which mostly females emerge from large host individuals and mostly males from small hosts. He suggests that this information may be applied advantageously in biological control work involving the insectary rearing of stocks for colonization, where the use of large hosts would not only produce a higher proportion of female parasites but also females with greater reproductivity capacity and thus permit a still more rapid increase of stocks for colonization purposes.

From the data presented by Clausen (15), it is clear that the sex ratio of a given parasitic species is not a fixed figure. Clausen concludes:

> It will vary (1) with the sex ratio of the host; (2) with successive generations upon the same or a different host generation; (3) with different hosts; (4) upon the same host and in the same season, but in different geographical regions; and (5) in successive years when the host population is increasing or declining rapidly.

Flanders (43) has noted that certain species such as *Pauridia peregrina* Timberlake and *H. rouxi* usually are unisexual, but occasionally numerous males will appear. Flanders (43) believes this bisexual uniparental development is facultative because the mechanism is gonadal and triggered by nutritive circumstances during cell division. His preliminary work showed that

temperature is involved, and he suggests that "the effect of temperature on the sexuality of uniparental Hymenoptera may be nutritional in that it affects the rate of metabolism." It is therefore clear that, while the sex ratio of a parasitic species may be varied by many environmental factors which favor the production of one sex over the other, an investigator soon finds himself back to the fundamental problem of sex determination in these parasitic species.

Sex Determination in Parasitic Hymenoptera

Whereas the eggs of most insects undergo meiosis and normally develop no further unless activated by fertilization, this is not the case with ants, bees, and wasps, for the entire order Hymenoptera is characterized by parthenogenesis. Among the parasitic Hymenoptera this development by parthenogenesis can be subdivided into three categories: thelyotoky, deuterotoky, and arrhenotoky.

Thelyotoky.—There are some species such as *I. canescens* which are obligatorily parthenogenetic and each generation consists entirely of females. Such a species in which males are virtually unknown is thelyotokous in the strictest sense, and all individuals are termed "impaternate" or "uniparental."

Deuterotoky.—There are other species, such as *H. rouxi* and *Tropidophryne melvillei* Compere, which normally exhibit thelyotoky, but also produce a few males. The occurrence of these exceptional males (spanandry) would classify such species as being deuterotokous or ampherotokous. Here again the individuals are all uniparental.

Arrhenotoky.—The majority of species of parasitic Hymenoptera exhibit facultative parthenogenesis, i.e., the eggs may develop either parthenogenetically or zygogenetically, depending upon the occurrence of fertilization. In these cases the fertilized eggs are diploid and give rise to females, whereas the azygotes from the unfertilized eggs are haploid and are males. This type of parthenogenesis is termed "arrhenotoky." In an arrhenotokous species the females are normally biparental and the males are termed uniparental or impaternate.

This type of genetic system, variously referred to as "haplodiploidy," "haploid parthenogenesis," or "arrhenotoky," is common among the parasitic Hymenoptera, and the rule that applies to such species is often termed Dzierzon's Law [Whiting (83, 84)]. It is so named because in 1845 Joannes Dzierzon, a Silesian priest and a keen student of honey bees, put forth the theory that drones develop from unfertilized eggs while the female workers and queens come from fertilized eggs. Dzierzon's theory stemmed from the observations that unmated and old queens produce drone broods and that in race crossing the drones are matroclinous while the daughters are hybrid. It is perhaps of more than passing interest to biologists that Dzierzon was aware of segregating heredity at least 12 years before the publication of Mendel's classic paper on peas [Whiting (83)].

Furthermore, it is evident that Dzierzon glimpsed the fundamental gametic ratio when in an 1854 paper he stated that the drones of the second generation of a cross resemble either the paternal or maternal race and that these two types occur in equal numbers.

Thus, in accordance with Dzierzon's Law, the usual pattern in parasitic Hymenoptera is that the eggs undergo reduction in chromosome number and thus become haploid as if in preparation for fertilization. If fertilization does not occur, then these eggs develop by parthenogenesis into haploid individuals of the male sex. Whiting (84) has stated that this "production of males by haploid arrhenotoky is a stable mode of bisexual reproduction functioning quite as successfully as the more frequent method of zygogenesis of both sexes." Furthermore, he says it "is highly perfected, reproductively economical, facultative in the highest degree." It is also generally agreed that this mode of reproduction does not lack the advantages of amphimixis.

White (81) states:

> [Arrhenotoky] is a method of sex determination as well as a form of reproduction, the frequency of males in the population being determined by the frequency with which unfertilized eggs are laid. . . . Forms in which sex determination depends on male haploidy will likewise be free to develop, through natural selection, whatever sex ratio is best adapted to their reproductive economy. Variable and fluctuating sex ratios are particularly characteristic of haplodiploid groups.

The maintenance of haploid arrhenotoky requires a combination of at least three conditions not generally found in animals: (*a*) survival of the azygote with reduced chromosome number, (*b*) a regulation of spermatogenesis so that the sperms of the haploid male retain the full haploid set of chromosomes, and (*c*) a rather special system of sex determination, i.e., one which may be different from that based on the idea of genic balance.

Viability of haploid males.— The viability of the haploid male is an exception to the general rule among animals, and a good discussion of this phenomenon in relation to haploid arrhenotoky is found in the work of Whiting (84). He puts emphasis on the essential retention of a satisfactory surface-volume relationship in the cells.

Greenshields (45) suggests that the males are viable because the apparent or presumed haploid set of chromosomes is fundamentally diploid. Since females have been shown to possess twice the male chromosome number, in certain tissues at least, it follows that the females would be tetraploid.

It was reported by Speicher & Speicher (72) that the occasional uniparental female appearing in *Bracon hebetor* Say originated from patches of tetraploid tissue in an ovary which was otherwise diploid, and the idea has been extended by Flanders (34) who believes that the occurrence of males in *Prospaltella perniciosi* Tower are the result of the existence of patches of diploid tissue in ovaries that otherwise are tetraploid.

Whiting (84) points out that there appears to be a somatic diploidy in the drone honey bee and that in some of the lower Hymenoptera there is

pairing, to some extent, among the chromosomes of the haploid set in the male. Theorizing from the broad biological basis that in polyploid plants the gametic number of chromosomes has been called haploid, Whiting (84) states:

> This haploid number may be various multiples of a more fundamental monoploid number which, presumably, acted as the haploid number of some ancestral type. The term haploid is, therefore, a relative term. It is not improbable that there may be remnants of genetic polyploidy in the lower Hymenoptera or in certain higher groups, but this has disappeared or does not exist at all in others. In any case the somatic regulative polyploidy of the bee appears to be a very different phenomenon.

Certainly the genetic data on *Bracon* (=*Habrobracon*) show that it breeds as a haploid. White (81) comments:

> In view of the widespread occurrence of endopolyploidy in insects it seems quite probable that many of the somatic tissues in haplodiploid forms are, in actual fact, highly polyploid in both sexes. They have been derived, however, from haploid and diploid cells in the male and female embryos. It is, unfortunately, not known whether the somatic nuclei of male and female haplodiploids reach the same degree of polyploidy in the course of development, or whether tissues which are "endoctoploid" in females are "endotetraploid" in males and so on.

White (81) further points out that in the parasitic wasp *Bracon* the somatic cells of the haploid males appear to be about the same size as those of the diploid females, but those of the diploid males are considerably larger [Grosch (46)]. Whiting (84) prefers to speak of the males of the Hymenoptera as "impaternate" (fatherless) rather than "haploid," in view of the presumed endopolyploidy of most of their somatic tissues.

Meiosis in the haploid male.—Another requirement for haploid arrhenotoky is that spermatogenesis be regulated so that the sperm cells will have the full haploid set. Since male Hymenoptera are haploid, it is clear that their spermatogenesis will be unlike the normal meiotic divisions in most other animals. White (81) believes that, despite all published accounts which would seem to indicate otherwise, there are actually only two main types of spermatogenesis to be found among the Hymenoptera. The traditional view has been that there is a failure of nuclear division in the first spermatocyte and instead of any splitting of chromosomes there is only the pinching off of a small cytoplasmic bud. The second division is equal, involving simple mitosis which results in the formation of two equivalent spermatids, each of which develops into a functional sperm. White (81, 82) points out that the difference between the spermatogenesis in the bees and the remaining Hymenoptera lies in the fact that the maturation division in the former is cytologically unequal and results in the formation of one large cell and one small one. Only the larger cells form functional sperms in the bees. Thus, whereas most Hymenoptera form two sperms from each spermatocyte, the bees form only one.

Sex determination in arrhenotokous species.—It is known that sex determination in many animals, e.g., *Drosophila,* depends upon genic balance, but

in haploid arrhenotoky sex determination appears to be fundamentally irreconcilable with this principle [White (81); Whiting (84)].

In their famous studies on *Bracon* (=*Habrobracon*), the Whitings found that closely inbred stocks produced some diploid males. Whiting (84) reviews the findings as follows:

> Recessive females crossed with dominant males produce recessive haploid sons and dominant heterozygous daughters, as expected according to Dzierzon's Law, but if the parents come from the same general stock, then there are also regularly produced diploid biparental males, which resemble their sisters in showing all the dominant traits inherited from both parents, but which are in no way intersexual. They are, however, of low viability and almost sterile. Their sperms are diploid, and consequently their few daughters are triploid.

Whiting (84) also stated that sex determination has been shown to depend upon a series of multiple alleles, of which nine have thus far been identified. These are designated *xa*, *xb*, *xc*, through *xi*. Any heterozygote (diploid), *xa/xb*, *xa/xc*, *xc/xd*, etc., is female; any azygote (haploid), *xa*, *xb*, *xc*, etc., or any homozygote (diploid), *xa/xa*, *xb/xb*, etc., is male.

In haplodiploid species multiple sex allelism may be the more primitive and general method of reproductive economy, for closely inbred species apparently have adopted some other method. Schmeider & Whiting (61) studied *Melittobia*, where close inbreeding is the natural habit. Here there were no diploid males even in the backcrossing of sons to mothers. As a matter of fact, outcrosses of *Melittobia* were found to be less fecund than close crosses.

Da Cunha & Kerr [see Kerr & Laidlaw (47)] are proponents of another hypothesis for sex determination in the Hymenoptera. They suggest that there are genes for femaleness (f) having a cumulative effect (ff=2F) and genes of maleness (m) which do not have a cumulative effect (mm=1M). Hence, sex can be determined according to the balance between these genes, as follows: haploid=M > F, and therefore the sex would be male; diploid =2F > M, and therefore the sex is female.

Clearly, no common pattern of sex determination exists in the parasitic Hymenoptera, and it is also evident that these insects offer a wonderful field for careful cytological study of this interesting aspect of their biology.

Most of the hypotheses on sex determination in the parasitic Hymenoptera need more experimental support and there are many problems still to be solved. One is how the diploid condition is maintained in thelyotokous species, and another is how intersexes originate in such insects [Doutt & Smith (25)]. More complete knowledge exists on the production of gynandromorphs than on the occurrence of intersexuality.

Rothenbuhler *et al.* (57) have shown that in honey bees the pronucleus of the matured egg unites with a spermatozoon to form a zygote and accordingly gives rise to diploid (female) tissues. However, since polyspermy is the rule, one (or more) of the supernumerary sperms which enter the egg may undergo cleavage independently to produce haploid male tissue. By

using female and male bees of known genetic constitution, it was shown that the male tissue on the gynandromorphs could have come only from the father. Furthermore, Rothenbuhler *et al.* (58) showed that when gynandromorphs had male reproductive organs the spermatozoa were functional and were of androgenetic origin.

Gynandromorphs may also occur if the egg pronucleus divides and one daughter nucleus unites with a spermatozoon while the other develops independently. In *Bracon*, gynandromorphs may be explained on the basis of the function of a recessive gene (gy). In other insects the cooling of fertilized eggs has produced gynandromorphs, and it is possible that the cooling interferred with the usual sequence of development and thereby resulted in a modification of some structures. As noted above, Flanders (43) has found temperature to be involved in the occurrence of males in deuterotokous species.

Mechanics of fertilization and regulation of sex ratio.—Obviously there is no problem of sex ratio in obligatorily thelyotokous species, and neither is there in practical work any concern expressed over the appearance of the males in deuterotokous species. However, the widely variable and often greatly fluctuating sex ratios of arrhenotokous species may cause difficulty in biological control projects both in the insectary culture and in the colonization of such parasites. This has been discussed briefly under the effect of host size on the sex ratio of the parasites, and it deserves a more thorough examination here.

Flanders (38) points out that when the spermatheca in arrhenotokous species contains spermatozoa it becomes a sex-changing mechanism. The sex of the egg is determined during oviposition, and the stimulation of the spermatheca to discharge spermatozoa into the oviduct is usually brought about by external conditions. Therefore, Flanders (38) concludes that the sex ratio in arrhenotokous species is variable because the action of the spermatheca is determined by inconstant environmental factors.

Flanders (38, 35) is convinced that the sperms stored in the spermatheca of the mated female are quiescent and must be subjected to some activating agent before they will migrate down the sperm duct to fertilize an egg. The source of the activating agent appears to be the spermathecal gland. Flanders (35) believes that this gland responds to external stimuli much as do the salivary glands in mammals.

There are many factors, both intrinsic and extrinsic, which influence the sex ratio of arrhenotokous species. Flanders (36) found that when the females of *M. ancylivorus* were subjected to repeated matings under insectary conditions a great preponderance of males occurred in their progeny. He (36, 35) reports:

> The *Macrocentrus* female, at each mating, receives a spermatophore from the male. After the spermatophore is adjusted so that it makes contact with the opening of the spermatheca and sperms have begun to move into the sperm capsule, a second

mating rarely if ever takes place. Females that mate before this adjustment is completed will mate many times. They receive so many spermatophores that none become adjusted and all are discarded before the female becomes impregnated. Multiple mating prolongs the preoviposition period and results in the production of progeny predominantly male.

In summary Flanders (35) states:

Sex ratios are determined *extrinsically* by the effect of the density of oviposition sites (hosts) on (a) differential mortality during development; (b) the premating interval after emergence, which determines the proportion of eggs deposited before mating and the inhibition of mating; (c) excessive mating; (d) the difference in oviposition response before and after mating; (e) the occurrence of preferred oviposition site; and (f) the rate of oviposition.

He also points out that sex ratios may be

. . . determined *intrinsically* by (a) the number of eggs deposited at one insertion of the ovipositor, (b) the number of ovarian eggs ready for deposition, and (c) the differential in the polyembryonic proclivity of the sexes.

Polyembryony.—The role of sex in governing the extent of polyembryonic division seems to have escaped attention until Flanders (30) suggested that the sexual differential in polyembryonic proclivity is a general phenomenon. He pointed out that among the Chalcidoidea the polyembryonic species tend to produce more individuals from fertilized than from unfertilized eggs. Doutt (23) studied 525 broods of *Copidosoma koehleri* Blanchard totalling 14,161 individuals. The mean number of individuals arising from an exclusively male brood was 21 and from a brood solely female, 31.4. It is interesting to note that a difference exists in the minimum number of individuals in broods of different sexes. Male broods may contain only eight individuals, whereas normal female broods were never observed to contain less than 17. Obviously, the presence of the sperm is the only fundamental difference between the fertilized and unfertilized eggs at the time of deposition. The sperm therefore influences the extent of the effective division, and presumably this is the product of the active chromatin-cytoplasmic ratio.

Furthermore, it appears that the presence of sperm in the egg is associated with the occurrence of the peculiar teratoid larvae. Doutt (24) found that these abnormal larvae are associated with female broods only. It is possible that their origin is androgenetic or that a supernumerary sperm unites with a polar body to produce this precocious larval type.

Nutrition

Trophamnion.—In polyembryonic species the trophamnion arises from the portion of the egg which contains the polar bodies, and its function is both protective and nutritive. While it thus has an essential role in polyembryonic species, it is no less important in some monoembryonic forms.

Smith (71) found that at eclosion of the egg of *M. vittatae* the trophamnion dissociates into component cells which neither degenerate nor lose their trophic function. Instead these cells float freely in the body of the host and increase tremendously in volume until they may be 3400 times the original size. They become filled with fat which is absorbed from the host beetle. These "giant cells" are the chief source of food for the parasite larvae in their later stages.

Host feeding.—Parasitism is a function only of the immature stages of parasitoids, but the adult females of some parasitic Hymenoptera do feed on the hosts also. This host feeding and host mutilation on the part of the female consititutes a form of predation [Flanders (39); DeBach (21)]. It is well established that this feeding on the body fluids of the host is necessary to obtain protein needed for ovigenesis.

Flanders (29, 39) has demonstrated that if *M. helvolus* is withheld from its host for three weeks at 80°F. ovigenesis ceases from lack of protein. When placed with its host, the female immediately starts feeding upon it and three days later begins to oviposit.

The protein needs in some species may be supplied by feeding on honeydew or on plant nectaries, both of which have been shown to contain free amino acids. There is a growing awareness of the role of honeydew in the longevity and fecundity of insect species. A compilation of much of this information is to be found in Zoebelein (85, 86, 87).

Ovisorption.—Flanders (28) has observed that in some parasitic Hymenoptera the production of eggs (ovigenesis) may be a more or less continuous process. If there are no hosts available to these females, their ripe eggs will be absorbed in the process known as ovisorption. Presumably this cycle permits the retention of metabolites and is thus physiologically economical in that it conserves reproductive material. While ovigenesis may require several days, the ovisorptive process may occur in a few hours [Flanders (42)]. This degeneration occurs in the ovariole and not in the oviduct.

According to Flanders (42), who is the foremost student of the subject, the extent to which the egg is absorbed varies with the species: the ovarian eggs of pteromalids disappear completely; those of certain encyrtids have only the egg content extracted. Flanders (42), writes:

> With *Encyrtus fuliginosus* Compere, for example, the collapsed shells of the absorbed eggs accumulate in the body cavity, having apparently been forced by the pressure of ovigenesis through the ovarial sheath. This penetration of the sheath can occur readily because the aeroscopic plate of the egg, when the shell is collapsed, forms a sharp, spinelike process.

Flanders (40, 42) believes that partial ovisorption may result in undernourishment of the embryo. He states (40):

> The ovisorption process by its graduated effect on the eggs (prior to the point of injury) allows for the fullest expression of the polymorphic potentialities inherent

in the germ plasm. This may result in the occurrence of caste intergrades in certain species of social Hymenoptera, whereas there are none in other species.

Respiration

Clausen (18) has compiled the published information on the respiratory adaptations found in the immature stages of parasitic insects. Earlier writers pointed out the similarity of the respiratory problems of aquatic and endoparasitic larvae, and the parallelism that exists in the manner by which the various obstacles presented by these two modes of life are overcome. However, Clausen believes this parallelism is more apparent than real, for some of the most common devices employed by aquatic larvae are not known to occur among those of endoparasitic habit.

Clausen (18) notes that many endoparasitic eggs which undergo extended embryonic growth possess a very thin, elastic chorion or on hatching are covered by a trophamnion. It is assumed that the oxygen requirements are absorbed from the host's blood through this membrane. Doutt (23) noted that the polygerms of *Copidosoma* are entwined in a network of host tracheae. The host apparently grows these tracheae into the area where the polygerms are situated in response to an oxygen deficiency in the surrounding tissue. The role of the aeroscopic plate in supplying the respiratory needs of encyrtid embryos and larvae has been described by Maple (52, 53).

As Clausen (18) has noted, there is relatively little definite information, based on adequate experimentation, regarding the respiratory needs and processes of parasitic larvae:

> In most instances the adaptations are assumed to have that function in the absence of any other apparent purpose, and though the assumption may be logical, yet there still remains the necessity for experimental proof.

For example, in the caudate larvae of certain ichneumonids and braconids the taillike appendage was once thought to be respiratory in function. Thorpe (73) by careful experimentation showed that it has no such function. It has also been suggested that the structure is an egg burster that aids in eclosion.

Venom

The parasitic Hymenoptera may or may not paralyze or kill their hosts prior to oviposition. Some species apparently never inject venom in the host, and in other species it is obviously essential that the host be killed or paralyzed before it is suitable for the development of the immature wasps. The host reaction to the venom may be immediate as when *Perisierola emigrata* Rohwer paralyzes a lepidopterous host, or the reaction may be delayed and no symptoms evident for several minutes as when *Liriomyza* is stung by *Solenotus begini* Ashmead.

Very little is known about the venom of parasitic Hymenoptera, and the work of Beard (6) is the first serious investigation of the subject. In

studying the venom of *B. hebetor,* Beard calculated that one part of venom in 200,000,000 parts of host blood was sufficient to cause permanent paralysis. The venom is transported by the hosts' blood to the site of action. Since the paralysis results from impairment of the excitatory processes of the body wall musculature, the site of action is considered to be the neuromuscular junction.

LITERATURE CITED

1. Allee, W. C., Emerson, A. E., Park, O., Park, T., and Schmidt, K. P., *Principles of Animal Ecology* (W. B. Saunders Co., Philadelphia and London, 837 pp., 1949)
2. Allen, H. W., *J. Econ. Entomol.*, **47,** 278–81 (1954)
3. Allen, W. W., and Smith, R. F., *Hilgardia* (In press)
4. Andrewartha, H. G., *Biol. Revs. Cambridge Phil. Soc.*, **27,** 50–107 (1952)
5. Ball, G. H., *Am. Naturalist,* **77,** 345–64 (1943)
6. Beard, R. L., *Conn. Agr. Expt. Sta. Bull., No. 562,* 27 pp. (1952)
7. Bess, H. A., *Ann. Entomol. Soc. Am.,* **32,** 189–226 (1939)
8. Bronskill, J. F., and House, H. L., *Can. Entomologist,* **89,** 483 (1957)
9. Bryden, J. W., and Bishop, M. W. H., *Entomologist's Monthly Mag.,* **81,** 51–52 (1945)
10. Burnett, T., *Ecology,* **29,** 181–89 (1948)
11. Burnett, T., *Ecology,* **30,** 113–34 (1949)
12. Burnett, T., *Am. Naturalist,* **85,** 337–52 (1951)
13. Burnett, T., *Ecology,* **34,** 322–28 (1953)
14. Burnett, T., *Physiol. Zoöl.,* **27,** 239–48 (1954)
15. Clausen, C. P., *J. N. Y. Entomol. Soc.,* **47,** 1–9 (1939)
16. Clausen, C. P., *Entomophagous Insects* (McGraw-Hill Book Co., Inc., New York, N.Y., 688 pp., 1940)
17. Clausen, C. P., *Psyche,* **48,** 57–69 (1941)
18. Clausen, C. P., *Arthropoda,* **1,** 197–224 (1950)
19. Clausen, C. P., *Boll. lab. zool. gen. e agr. Portici,* **33,** 119–33 (1956)
20. Clausen, C. P., Jaynes, H. A., and Gardner, T. R., *U. S. Dept. Agr. Tech. Bull., No. 366,* 58 pp. (1933)
21. DeBach, P., *J. Econ. Entomol.,* **36,** 647–58 (1943)
22. Dethier, V. G., *J. Exptl. Zool.,* **105,** 199–208 (1947)
23. Doutt, R. L., *Am. Naturalist,* **81,** 435–53 (1947)
24. Doutt, R. L., *Can. Entomologist,* **84,** 247–50 (1952)
25. Doutt, R. L., and Smith, R. A., *Can. Entomologist,* **82,** 165–70 (1950)
26. Finney, G. L., Flanders, S. E., and Smith, H. S., *Hilgardia,* **17,** 437–83 (1947)
27. Flanders, S. E., *Univ. Calif. Publs. Entomol.,* **6,** 401–22 (1937)
28. Flanders, S. E., *Ann. Entomol. Soc. Am.,* **35,** 251–66 (1942)
29. Flanders, S. E., *J. Econ. Entomol.,* **35,** 690–98 (1942)
30. Flanders, S. E., *J. Econ. Entomol.,* **35,** 108 (1942)
31. Flanders, S. E., *J. Econ. Entomol.,* **36,** 807 (1954)
32. Flanders, S. E., *J. Econ. Entomol.,* **36,** 802–3 (1943)
33. Flanders, S. E., *J. Econ. Entomol.,* **37,** 408–11 (1944)
34. Flanders, S. E., *Science,* **100,** 168–69 (1944)
35. Flanders, S. E., *Quart. Rev. Biol.,* **21,** 135–43 (1946)
36. Flanders, S. E., *J. Econ. Entomol.,* **38,** 323–27 (1946)

37. Flanders, S. E., *Hilgardia*, **21**, 1–42 (1951)
38. Flanders, S. E., *Ann. Entomol. Soc. Am.*, **32**, 11–26 (1952)
39. Flanders, S. E., *J. Econ. Entomol.*, **46**, 541–44 (1953)
40. Flanders, S. E., *Sci. Monthly*, **76**, 142–48 (1953)
41. Flanders, S. E., *J. Econ. Entomol.*, **46**, 266–69 (1953)
42. Flanders, S. E., *Sci. Monthly*, **85**, 176–77 (1957)
43. Flanders, S. E. (Unpublished notes, 1957)
44. Gayspitz, K. F., and Kyao, I. I., *Entomol. Oboz.*, **33**, 32–35 (1953)
45. Greenshields, F., *Nature*, **138**, 330 (1936)
46. Grosch, D. S., *Growth*, **9**, 1–17 (1945)
47. Kerr, W. E., and Laidlaw, H. H., Jr., *Advances in Genet.*, **8**, 109–53 (1956)
48. Laing, J., *J. Animal Ecol.*, **6**, 298–317 (1937)
49. Lees, A. D., *The Physiology of Diapause in Arthropods* (Cambridge University Press, Cambridge, England, 150 pp., 1955)
50. Lees, A. D., *Ann. Rev. Entomol.*, **1**, 1–16 (1956)
51. Lloyd, D. C., *Bull. Entomol. Research*, **42**, 707–20 (1952)
52. Maple, J. D., *Ann. Entomol. Soc. Am.*, **30**, 124–54 (1937)
53. Maple, J. D., *Univ. Calif. Publs. Entomol.*, **8**, 25–122 (1947)
54. Muldrew, J. A., *Can. J. Zool.*, **31**, 313–32 (1953)
55. Narayanan, E. S., and Chaudhuri, R. P., *Bull. Entomol. Research*, **45**, 647–59 (1954)
56. Parker, H. L., *U. S. Dept. Agr. Tech. Bull., No. 477*, 17 pp. (1935)
57. Rothenbuhler, W. C., Gowen, J. W., and Park, O. W., *Genetics*, **36**, 573 (1951)
58. Rothenbuhler, W. C., Gowen, J. W., and Park, O. W., *Science*, **115**, 637–38 (1952)
59. Salt, G., *Proc. Roy. Soc. (London)*, [B]**117**, 413–35 (1935)
60. Salt, G., *Biol. Revs. Cambridge Phil. Soc.*, **16**, 239–64 (1941)
61. Schmeider, R. G., and Whiting, P. W., *Genetics*, **32**, 29–37 (1947)
62. Schneider, F., *Mitt. schweiz, entomol. Ges.*, **23**, 155–94 (1950)
63. Schneider, F., *Z. angew. Entomol.*, **33**, 150–62 (1951)
64. Schneiderman, H. A., *Physiol. Triggers*, **1**, 46–59 (1956)
65. Simmonds, F. J., *Rev. can. biol.*, **2**, 15–58 (1943)
66. Simmonds, F. J., *Bull. Entomol. Research*, **37**, 95–97 (1946)
67. Simmonds, F. J., *Can. Entomologist*, **79**, 226–32 (1947)
68. Simmonds, F. J., *Phil. Trans. Roy. Soc. (London)*, [B]**233**, 385–414 (1948)
69. Skoblo, I. S., *Compt. rend. acad. sci. U. R. S. S.*, **33**, 424–26 (1941)
70. Smith, J. M., *Can. Entomologist*, **89**, 219–30 (1957)
71. Smith, O. J., *Univ. Calif. Publs. Entomol.*, **9**, 315–44 (1952)
72. Speicher, K. G., and Speicher, B. R., *Biol. Bull.*, **74**, 247–52 (1938)
73. Thorpe, W. H., *Proc. Roy. Soc. (London)*, [B]**109**, 450–71 (1932)
74. Thorpe, W. H., and Jones, F. G. W., *Proc. Roy. Soc. (London)*, [B]**124**, 56–81 (1937)
75. Ullyett, G. C., *J. Entomol. Soc. S. Africa*, **6**, 65–80 (1943)
76. Ullyett, G. C., *Union S. Africa Dept. Agr. Entomol. Mem.*, **2**, 77–202 (1947)
77. Ullyett, G. C., *Entomol. Soc. S. Africa Mem.*, **2**, 1–89 (1953)
78. Varley, G. C., and Butler, C. G., *Parasitology*, **25**, 263–68 (1933)
79. Wallace, G. E., *Ann. Carnegie Museum*, **29**, 31–38 (1942)
80. Wardle, R. A., and Buckle, P., *The Principles of Insect Control* (Manchester University Press, London, England, 295 pp., 1923)

81. White, M. J. D., *Animal Cytology and Evolution*, 2nd ed., (Cambridge University Press, Cambridge, England, 454 pp., 1954)
82. White, M. J. D., *Ann. Rev. Entomol.*, **2**, 72–90 (1957)
83. Whiting, P. W., *J. Heredity*, **26**, 263–78 (1935)
84. Whiting, P. W., *Quart. Rev. Biol.*, **20**, 231–60 (1945)
85. Zoebelein, G., *Z. angew. Entomol.*, **38**, 369–416 (1956)
86. Zoebelein, G., *Z. angew. Entomol.*, **39**, 129–67 (1956)
87. Zoebelein, G., *Forstwiss. Centr.*, **76**, 1–64 (1957)

BIOCLIMATIC STUDIES WITH INSECTS[1]

By P. S. Messenger
Department of Biological Control, University of California, Albany, California

Weather and climate are commonly accepted by entomologists as dominant influences on the behavior, abundance, and distribution of insects. The literature on the subject of climate and insects is extraordinarily voluminous, as may be seen by perusal of most texts on animal ecology, a recent example being the large volume by Andrewartha & Birch (7). Specific phases of this field, such as the influence of climate and weather on behavior and on outbreaks of pest insects have been reviewed recently by Graham (52) and Wellington (194). With the increasing use of the ecological approach in insect zoogeography [Hesse *et al.* (64); Gressitt (54)], it is appropriate to consider now the relationships between climate and the distribution of insects, a major field of insect bioclimatics.

Hopkins (68) has broadly defined "bioclimatics" as the science of life and climate relations. For economic entomology, the subject matter of the field and the manner in which the bioclimatic characteristics of a species might best be determined have been well outlined by Uvarov (184). For the purposes of this review, the subject matter is divided into the following aspects: biological, which deals with laboratory studies of effects of the various climatic factors on insects and with methods for determining insect distribution and abundance in nature; climatological, which treats the recording and measuring of the various climatic factors in areas where the species occurs and the methods for analyzing and comparing climatic data; bioclimatic correlations, which concern the methods by which climate is found to control insect distribution; and bioclimatic forecasting, which uses bioclimatic analysis in predicting the potential spread of a species into uninfested areas.

So far as the physical environment is concerned, extension in range of a species is considered limited by two broad categories of controls, geographic barriers and climatic restrictions. In the case of agriculturally and medically important insect species, the former limitation, geography, is rapidly losing importance because of the activities of mankind [Smith *et al.* (169), Adamson (1)]. Therefore, many injurious species are no longer limited strictly to their primitive ranges or to their primitive balances with the environment. Because of this, the bioclimatic aspects of insect distribution and abundance have commanded an increasing amount of attention during the past half-century. The continuing importance of such efforts has been emphasized repeatedly [Shelford (155, 156); Uvarov (184); Sweet-

[1] The survey of the literature pertaining to this review was completed in June, 1958.

man (175); Lukyanovich (111); Rubtzov (144)], with more recent reemphasis by Uvarov (186), Williams (197, 198), Mills (124), and Wellington (193), among others.

Biological Aspects of Insect Bioclimatics

Since laboratory studies of the effects of isolated, carefully controlled factors of climate on insects have been well summarized by Shelford (158), Uvarov (184), Chapman (27), and Allee *et al.* (3), as well as Andrewartha & Birch (7), only the contributions more recently used in bioclimatic analyses and those influencing or modifying current bioclimatic methods are treated here.

Temperature.—Because the factor of temperature has been considered the dominant one controlling insect development and survival, many bioclimatic studies with insects have been based in large part on some measure of temperature as a limiting factor in insect distribution. Bioclimatic analyses have made use of temperature thresholds, thermal constants, optimal thermal conditions, and lethal levels of this climatic factor.

Laboratory studies show that the temperature threshold for insect development is not a completely stable biological characteristic, but is modified by acclimatization, variations in temperature during exposure, environmental factors other than temperature, and stage of development of the individual [Rubtzov (144); Lin *et al.* (105)]. The threshold, commonly applied to initiation of development in the embryonic stage, now is applied also to many different life processes and activities and to all stages of development. Paralleling the discovery that the threshold is a somewhat variable characteristic are those studies showing that the thermal constant, or indicator of the heat required to complete development, is also an unstable property, modified by acclimatization, the temperature levels involved during exposure, and other environmental factors [Shelford (158); Rubtzov (144); Kozhanchikov (94, 95, 99)].

The thermal optimum, or temperature level most favorable for the species, needs reconsideration in the light of recent studies. Effects of temperature on the physiological processes within the insect indicate that thermal levels leading to fastest development or to highest survival may not necessarily be the most suitable conditions for the species [Kozhanchikov (93, 94, 98)]. One manner of arriving at an estimate of the thermal optimum is to base it on the maximum rate of increase of entire population samples, rather than individuals [Howe & Burges (70)]. Further, it should not be automatically assumed that optimal conditions of temperature, humidity, and light as found in laboratory studies will also apply to the species in its actual environment. A number of studies show that the greatest abundance of a species may occur in climatic environments not considered optimal on the basis of laboratory experiments [Rubtzov (143); Michelbacher & Leighly (123); DeBach *et al.* (42)].

The use of constant temperature results, determined in the laboratory,

for analyzing climatic influences in the field may also require modification. A very important factor in insect bioclimatics is the quantitative relationship between constant and variable temperatures as they affect insect development and survival. Relatively recent observations of the effects of naturally fluctuating temperatures indicate that, at lower thermal levels, growth is accelerated over that which would be predicted on the basis of constant temperature conditions [Rubtzov (142), Stellwaag (172); Noll (127); Harries (59); Pradhan (133)]. At intermediate thermal levels, development appears to proceed at about the same rates as would be expected relative to constant temperatures exposures [Kozhanchikov (100); Munger & Cressman (125], while at the higher thermal levels, growth is usually retarded (100, 133). However, even the methods by which variable temperature effects are compared with these from constant temperature exposures are open to criticism [Ryan (147)]. The temperature-velocity curve for development cannot be considered linear [Janisch (75); Belehradek (12); Davidson (41); Pradhan (134)]. Hence, use of the arithmetic mean of the variable temperatures in comparing variable with constant temperature effects may lead to faulty conclusions. Subthreshold exposures cannot be ignored, as they commonly are, and as the thermal-constant and temperature-accumulation rules require [Ludwig & Cable (110); Powsner (132); Bucher *et al.* (24); Lin *et al.* (105)]. There is also some indication, briefly reviewed by Harries (60), that different times spent at different temperatures may not be presumed additive in contributing toward developmental endpoints, a presumption fundamental to the thermal constant theory.

Humidity.—Many observations on the resistance of a species to dryness or to desiccation show high correlations with the moisture conditions of the natural habitat of the species [Talbot (178); Ludwig (107, 108); Teterovskaya (180); Cloudsley-Thompson (30, 31)]. The Mexican bean beetle, *Epilachna varivestis* Mulsant, can hibernate successfully over the winter only in moist, humid habitats, while high humidities combined with high temperatures are destructive to larvae and pupae [Sweetman (173, 174)]. Both of these general results are considered important factors limiting the distribution of this beetle in North America.

Very little information is available on the influence or importance of continuously varying humidities, such as occur naturally, on insects. Most field observations on distribution are usually correlated with mean humidity levels. However, low, variable humidities stimulate the production of more *Epilachna varivestis* eggs than higher humidity levels, a result in direct contrast to that which occurs with this beetle under constant humidity conditions [Sweetman & Wedemeyer (177)]. Females of the parasite *Macrocentrus ancylivorus* Rohwer oviposit more freely when the humidity is high, a response that tends to impart a rhythmic, diurnal variation in rates of parasitism [Martin (117)].

Temperature and humidity combined.—These two factors show high degrees of interaction when affecting insect activity, development, and survi-

val simultaneously, as necessarily happens in nature. Shelford (158) and Andrewartha & Birch (7) demonstrate how temperature and humidity together affect reproduction and development of some species differently than does either factor alone. Bodenheimer (20) and Gunn (55, 56) show that humidity affects the temperature preference of various species, while Cloudsley-Thompson (30) and Platt *et al.* (131) show that temperature can modify the humidity preference of other species. The thermal death point may be lowered by low ambient humidities in some cases, while it may be raised in others [Ludwig (109)], and in certain instances the death point can be both raised and lowered in one and the same species, depending on the degree of dryness and the time of exposure [Guthrie & Decker (57); Roan (141].

Because of the interacting influences of temperature and humidity on insect behavior, development, and survival, many attempts have been made to find some other meteorological factor, or some single-valued statistic that will indicate their combined effects. One such meteorological factor is evaporation power of the air, influenced by temperature, relative humidity, and wind velocity. Lathrop (103) early used this factor as a measure of the influence of different humidity conditions on insect development and survival. Wellington (189, 190, 191) has found evaporation a better indicator of the influence of controlled variations in temperature and humidity on insect behavior than either of the controlled variables alone.

However, evaporation data are neither commonly provided by the usual weather reporting services, nor satisfactorily computed from the meteorological information that is provided. Since bioclimatic analysis requires information about the distribution of climatic indicators, which in this case is evaporation, over broad areas of the land, it has been necessary to infer the level of this factor from what data are available. One such method for estimating the levels of evaporation existing at different points uses the easily computed saturation-deficiency level of the air. This measurement has been shown in the laboratory to be closely related to the evaporation of moisture from insects in controlled humidity environments [Anderson (4); Buxton (26)]. But, use of saturation deficiency as a direct measure of evaporation has been criticized on physical grounds by Leighly (104) and Thornthwaite (182), and the latter worker explains how it may be possible to have different amounts of evaporation occurring under conditions of the same saturation-deficiency level. As will be shown below, many bioclimatic analyses relating insect distribution and climate have been based in some measure on the use of saturation deficiencies as indicators of the moisture influences, and care will be required in evaluating the suitability of such analyses.

Other environmental factors.—Some observations indicate that winter rain increases the lethal effects of winter cold on insects [Sanderson (149); Gjullin (50); Kozhanchikov (96)], while snow usually provides a protection against lethal air temperatures in the case of subterranean stages of

insects [Mail (113, 114); Sacharov (148)]. Rainfall is a major factor controlling the moisture content of soil, and therefore can be a major factor limiting the development and survival of such soil-inhabiting forms [Cook (34); Fox (47)]. Studies of the survival, location, and depth of pupation of the two tropical fruit flies *Dacus dorsalis* Hendel and *Dacus cucurbitae* Coquillett show that definite levels of soil moisture are required for successful pupation and adult emergence [Shibata (160, 161, 165, 166)]. This requirement is probably a major limitation on the spread of these species into the semiarid areas at the western extremities of their natural range in southern Asia.

Many studies show that insect behavior can be regulated by light intensity [Fraenkel & Gunn (48); Wellington (194)]. Such diurnal responses to light may have definite adaptive advantages for many species [Wellington (188, 195); Cloudsley-Thompson (29); Green (53)]. Most important for bioclimatic analysis is the possibility that different measures of a climatic factor limiting some particular activity will be brought into play, depending upon diurnal rhythms imparted by light to this activity. For example, insects that oviposit only at night are restricted in this activity by nocturnal temperatures, the best statistical measure of which would be the mean minimum daily temperatures encountered in a locality or in a season. Twilight activity would be under the control of late-afternoon temperatures, the best measure of which is the mean daily temperature.

Distribution and abundance records.—For purposes of bioclimatic analysis, the proper evaluation of distributional records and the limits of current distribution require defining. Cook (34, 35) specifies the limits of distribution on the basis of economic damage. Outside the zone of economic damage, Cook assumes, the insect may be present in low numbers and in spotted habitats, but not dominated by the general climate so much as by local variations in the microhabitats caused by differences in exposure, soil, and other physiographic and edaphic factors.

Records indicating that a species exists in certain localities, especially along the periphery of the range of the species, require critical evaluation. In some cases a species may be carried outside of its climatically limited range, either because of seasonal differences in climate, or because of world trade. For example, the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), has long been recorded from the vicinity of Paris, France [Bodenheimer (23)]. In fact, some of the limiting climatic conditions for this species, used in a bioclimatic analysis by Gjullin (50), were taken from the climate of Paris. But whether the species actually is endemic in the Parisian region or whether its presence there merely results from the importation of infested fruits from more southerly areas is not clear. Certain recent researches in France by Blanck (13) and Aubin (9) indicate that perhaps the latter is the more probable answer.

Proper taxonomic identification is also important. Confusion of the distributional records of the alfalfa weevil, *Hypera postica* (Gyllenhal) with

the closely related, thermophilic species *Hypera brunneipennis* Boheman temporarily rendered inexplicable the limited distribution of the former species in the somewhat cooler regions of California [Michelbacher & Leighly (123)]. Modern synonomy, taxonomic revisions, and the splitting of a given species into two or more species, while retaining the name of the original, can confuse the record for bioclimatic analysis.

Climatic Analysis

Bioclimatic analysis of insect distribution and abundance requires meteorological information for two purposes: (*a*) to determine what climatic factors, and what intensities of these factors, are limiting in the insect habitat, and (*b*) to ascertain how these factors, and their limiting levels, are distributed over the surface of the land. The former demands investigation of the climatic factors in the insect environment; the latter, a study of the distribution and classification of climates and climatic factors.

Climatic observations.—Recent discussion has re-emphasized that use of standard meteorological information, collected in standardized ways, is somewhat unrealistic in attempts to relate climate to insect behavior and survival [Smith (170); Wellington (194)]: the different climatic factors vary in intensity within limited distances in the natural environment. Such discussion points up the desirability of relating climate and insect ecology at the microenvironmental level.

On the other hand, while the insect microenvironment might for these reasons remain the climatic level of choice for carrying out a bioclimatic analysis, the paucity of general, widespread meteorological information of such a specialized nature and the almost complete lack of knowledge of the geographical distribution of the climatic factors in such restricted spatial limits require use of the usual, readily available meteorological data recorded at the standard shelter level. What is really needed is knowledge of the similarities and differences existing between the climatic factors at these two environmental levels. For example, studies by Cloudsley-Thompson (30, 31, 32), Green (53), Henson & Shepard (63), and Wellington (192) show the variations existing between certain climatic factors in particular insect microhabitats and in near-by weather shelters. Such comparisons have been of only very short duration. Attempts to correlate trends between factors of the microclimate and the macroclimate over long periods of time or over wide areas of land are rare. One example of such an attempt, however, is given by Haufe & Burgess (62), who demonstrate the possibilities in this regard in their treatment of the effects of meteorological conditions on the microenvironment of mosquito larvae and pupae.

Climatic indicators.—In describing the limiting or controlling influence of climate on distribution of plants and animals, many biologists have made use of such climatic indices as mean annual or seasonal isotherms, annual isohyets, or mean annual humidity levels. Such climatic statistics are open to criticism in that arithmetic means of such long-term periods are "fic-

tional," have no true relation to the actual causal factors dominating distribution, and are purely static aspects of climate [Davidson (38); Nomura (128); Lindroth (106)].

On the other hand, bioclimatic analysis gains much from the methods by which climatologists classify and locate the different climates of the world. Insect species will not be found limited to the climatic zones of the climatologists, but the sources of information, and many of the methods used to define, quantify, and delineate such climatic zones will prove highly useful in devising bioclimatic regions or in analyzing the bioclimatic characteristics of a particular insect.

The classification and distribution of climates has long been the subject of study by climatologists [Trewartha (183)]. Fortunately for bioclimatics, most of the recent systems devised by climatologists are based in some degree on certain biological features of the earth's surface—in particular, floristic distributional patterns [Köppen (92); Thornthwaite (181); Prescott (138); Trewartha (183)]. For example, the Köppen system of classifying climates is based on the important factors of temperature and moisture as major limiting factors in plant growth, and hence climates are defined according to this system on the basis of annual and monthly mean temperatures and precipitation totals. The Thornthwaite system accepts the basic premise of the thermal and moisture control of plant growth, but more accurately portrays the underlying mechanism of their controlling action by defining climatic zones on the basis of precipitation-effectiveness indices (ratios of precipitation to evaporation) and thermal-efficiency indices (ratios of temperature to evaporation), with minor divisions of climates based on seasonal concentration of rainfall. Various other measures of the interacting effects of temperature and moisture on general plant distribution have also been considered, such as the aridity index of Martonne (118) or the Meyer ratio of precipitation to saturation deficiency (122), which was used by Prescott (137, 138) to zone the climates of Australia.

Hence, in various schemes devised by biologists for defining the regional influence of climate on the distribution of life forms, use has been made of some of these climatic indices of the climatologists. The Life-Zone system of Merriam (119) and the Bioclimatic-Zone system of Hopkins (67) both make use of seasonal thermal indices. Davidson's Bioclimatic Zones of Australia are based on precipitation-evaporation ratios combined with various thermal and seasonal indices (39). A geographic set of co-ordinates based on thermal and moisture indices is used by Rubtzov (144) for defining distributional zones in Eurasia.

Climatic analysis.—Before the climates of different localities or regions can be compared with each other, some means of representation of the important, or dominant, climatic factors must be used or devised. Early methods for comparing climatic influences on insects made use of mean annual temperatures, humidities, or precipitation levels [Mansfeld (115); Holdaway (66); Predtetchenskii (136); Tehon (179)]. Such climatic indicators

are still much in use [Quilis Perez (140); Pepper (129); Koidsumi (90); Nash (126)].

In many instances, it has proven advantageous to compare more dynamic measures of different climates or environments, such as the annual march of temperature, humidity, and precipitation [McPhail & Bliss (112); Chaudry (28)]. Seasonal levels of the dominant climatic factors, essentially the basis of the thermal indices used by Merriam to define his Life Zones, have been used many times in insect bioclimatics [Sanderson (150); Rubtzov (144); Koidsumi (90)]. An especially useful method for displaying, analyzing, and mapping seasonal variations in temperature has been developed by Hartshorne (61), which may prove useful in insect bioclimatics.

For analysis of combinations of climatic factors, the climatograph method of combining measures of temperature and humidity, or the hythergraph for similar combinations with precipitation levels, has been the method of choice among a majority of insect ecologists. Various modifications of the basic climatograph have been proposed and applied: the bioclimatograph of Uvarov (185) includes a temporal representation of the developmental stages of the species being studied, and the ecoclimatograph of Bodenheimer (21) displays the favorable and limiting levels of the climatic factors under treatment. Combinations of the climatograph with the more detailed biological response curves (ellipses) of Pierce (130) or Shelford (157, 158), may be an improvement on the ecoclimatograph technique [see, for example, Howe & Burges (70)].

Aside from the comparison of climatographs by mere juxtaposition and overlapping onto biological response curves, the actual shapes and "directions" of the climatographs or hythergraphs may be analyzed and related to different climatic and vegetative environments [Smith (168)]. When more than two meteorological or environmental factors must be considered simultaneously, recourse may be taken to the polygonal graphing technique of Hutchinson (72), wherein limiting and favorable intensities of the different factors are plotted on radii of a circular graph. The resulting points, when joined by lines, form polygons that describe minimal, medial, and maximal environmental conditions. Similarities and differences among climates are then determined by comparison of these polygons.

Bioclimatic Correlations

With knowledge from laboratory studies of how insects are influenced in their development, survival, and reproduction by controlled factors of climate, coupled with information as to the manner in which the different climates and climatic factors are distributed geographically, the next obvious step is to attempt to find correlations between these two phases of bioclimatics and to ascertain how the controlling factors of climate appear to limit insect distribution. Different species, with separate geographical origins, and correspondingly distinct types of life cycles, are found to be limited in spread by different factors of climate, and by different measures of one

and the same factor. In this section, the various examples of such bioclimatic correlations are segregated according to the kinds of climatic indicators that appear to describe best the dominating factor limiting distribution. This should not be taken to imply that, where one particular climatic indicator has been used for relating the distribution of a species with climate, it is the only aspect of climate that the investigator has analyzed or found dominant. In many cases, several indicators have been described for a given species, and perhaps the most detailed bioclimatic analyses would make use of all possible climatic indices for delineating insect distribution and abundance.

The thermal constant as an index of distribution.—In response to the stimulus of Merriam's (119) Life-Zone system, many attempts were made to correlate the distribution of individual insect species with the amount of heat available at different climatic localities during the developmental or growing period of the life cycle. The basis for this type of analysis is the thermal constant theory of Sanderson & Peairs (152), which in turn derives from the presumed hyperbolic relationship between duration of development and temperature. The thermal constant and the threshold of development are physiological characteristics of a species easily determined in the laboratory. The major premise in using the thermal constant as a climatic indicator of distribution is that a species will find a satisfactory climatic environment in any locality warm enough to allow the accumulation of a sum of daily temperatures above the developmental threshold equal to the thermal constant.

By use of the thermal constant and the mean monthly temperatures for various different localities, the Mediterranean fruit fly, *Ceratitis capitata,* has been shown to be confined to countries where the climate permits at least two generations per year and where growth is interrupted by winter cold for no longer than about 100 days [Bodenheimer (14, 15)]. The number of generations of such multivoltine insects occurring at any locality can also be determined according to the amount of heat available during the year. Several other examples are known in which distribution is limited to areas where the local sums of effective temperatures equalled or exceeded the thermal constant for the respective species [Bodenheimer (16, 17); Kozhanchihov (97)].

In other studies of the distribution of insects and climate, the correlation between poleward range and summer isotherms [Predtetchenskii (136)] or between the increase in number of generations per year with increase in mean annual isotherms [Tehon (179)] is probably an indirect measure of the actual availability of heat, for which a better index might be the thermal constant.

From these results it would appear that the less heat required to complete development (i.e., the lower the thermal constant), the more poleward the distributional range should lie. However, the distribution of the two related species, *Sitophilus oryzae* (Linnaeus), and *Sitophilus granarius* (Linnaeus), cannot be so correlated: the more northerly species, *S. granarius,* has the higher thermal constant [Bodenheimer (18)]. This species develops

at a faster rate at low temperatures, while the more southerly *S. oryza* develops more swiftly in warmer environments. Similar results have been found for the distribution of related species of sawflies (*Diprion* spp.) in western Europe, with the species developing in the shortest time emerging earlier in the spring, and existing further north than other species under consideration [Elens (43, 44, 45)].

Hence, it becomes necessary to consider not only the magnitude of the thermal constant, but also the shape and location of the temperature-development curve. In the northern hemisphere, the order of occurrence from the north and the respective thermal constants for the following dipterans have been found to be: *Scopeuma stercorarium* (Linnaeus), 372 day-degrees; *Haematobia stimulans* (Meigen), 189 day-degrees; and *Siphona irritans* (Linnaeus), 141 day-degrees [Larsen & Thomsen (102)]. The first listed is therefore the slowest to develop while the last grows most rapidly under any given set of temperature conditions. However, the first is also able to develop at much lower temperatures than the others, as indicated by their respective thresholds of development: 2.1°, 10.7°, and 12.9°C. The slow growth rate of *S. stercorarium* coupled with its higher tolerance to cold conditions, allows it to survive in more northerly environments.

From our current knowledge of the thermal constant as a measure of the heat required for satisfactory development, its use as a limiting factor in insect distribution may be challenged on several points [see also criticisms of the Life-Zone concept, Kendeigh (76); Shelford (159)]. In many cases, there may be shown localities north of the existing range of a species where the climate is warm enough to permit the complete development of a generation [Sanderson (150); Rubtzov (146)], and consequently the limiting factor for poleward distribution must be some other aspect of temperature, or some other factor of the physical environment. As mentioned in an earlier section, the constancy of the thermal constant has also been questioned [Sanderson (151)], especially in connection with naturally fluctuating temperatures or other factors of the environment. Further, the use of only the developmental phases of an insect's life cycle for determining climatic limits is open to criticism [Davidson (40); Bodenheimer (21)]. Temperature effects on adult maturation, longevity, and reproduction must be considered. Only when all such factors are considered, and proper allowances and corrections are applied to the thermal constant, may an estimate of the geographical distribution and abundance of a species be determined on the basis of heat availability [Prebble (135); Bodenheimer (23)].

Winter cold as a limiting factor.—In temperate regions, one of the most apparent controlling factors of the environment on distribution is the cold of winter. In cases of species of temperate origin, the life cycle usually includes some physiological mechanism (such as diapause, hibernation, seasonal variations in cold-hardiness) or behavioral response (migration) that permits survival over what would otherwise be a lethally cold winter period. Species of tropical or subtropical origin, which breed continuously through-

out the year and are usually unequipped with such mechanisms, are, when present in temperate regions, influenced by cold winter temperatures more than any other climatic factor.

For many species of economically important insects in the northern hemisphere the northern limits conform quite closely with winter isotherms. For example, the poleward limits of the harlequin bug, *Murgantia histrionica* (Hahn), the corn earworm, *Heliothis zea* (Boddie), and the San Jose scale, *Aspidiotus perniciosus* Comstock, were found early in the century to conform to the winter isothermal pattern in North America [Sanderson (150)]. Sanderson pointed out that isotherms representing the lowest minimum temperature of record, the average annual minimum temperature, and the highest annual minimum temperature all more or less parallel each other (they will also be found to parallel rather closely the mean annual temperature), and hence any one of these isotherms could be used to delineate distribution. However, he considers the average annual minimum temperature a more meaningful measure of the limiting effects of winter cold, since the absolute annual minimum temperature occurs only at infrequent intervals (and possibly only once), while the maximum annual minimum and the annual mean temperatures overlook many important, short periods of lethal cold.

Laboratory-determined lethal temperature levels for a species may lead to inaccuracies when used to evaluate distribution in the field. Overwintering larvae of the European pine shoot moth, *Rhyacionia buoliana* (Schiffermüller), are killed in the laboratory by exposure to --28°C. [West (196)], and economic infestations are considered limited to areas south of the mean annual minimum isotherm −23°C. [Craighead (36)]. But, damaging populations have been found to occur in areas where minimum winter temperatures for a considerable number of years in succession fell below −28°C., a phenomenon not explained by variations in cold-hardiness [Batzer & Benjamin (11)].

Solomon & Adamson (171) discuss the problem of stored-products insect pests in temperate climates, finding that considerable differences in cold-hardiness and acclimatization ability exist among different species, and even in races and strains of the same species. Ability of different species to overwinter in British climates appears to a great extent related to the original habitat; those species from hot climates are the most susceptible to winter cold, while the hardy groups originated in temperature regions or have been long established outdoors there. Different aspects of winter severity are considered, such as the lowest temperature reached, length of periods below certain temperature levels, and the suddenness or gradualness of the onset of the cold period. The choice of a suitable criterion for evaluating winter severity must depend on the biology of the species concerned.

As an example, Burges (25) evaluated the efficiency of various climatic indicators in determining the favorability of British winters for the almond moth, *Ephestia cautella* (Walker), which overwinters in the larval stage.

Both laboratory and field observations on moth biology indicated that variable low temperatures are less deleterious to survival than constant conditions. The best measure of potential winter survival was the number of daily minima falling below 5°C. The selection of lower temperature levels, for example 2°C. or 0°C., failed to separate lethal from nonlethal winters. Use of mean minimum temperatures for the coldest month or of the three coldest months, or use of the number of weekly mean minima below 5°C., gave less satisfactory results since these time intervals have no direct relation to the life cycle of the species.

Summer temperatures as limiting factors.—Thermal conditions of the summer season may limit insect distribution by not providing sufficient warmth for normal reproduction and development (see section on thermal constant, p. 191) and also by interfering with normal activities or by causing high mortality as a result of excessively hot temperatures [Uvarov (184)]. Further, species with obligatory diapause or hibernation phases in their life cycles may be prevented from developing normally when exposed to continuously warm, though not lethal, temperature conditions such as occur in the tropics [Pepper (129)].

Summer heat alone is rather difficult to evaluate in restricting insect distribution, since in many regions excessively hot temperatures are closely correlated with aridity or extremely low humidities. In such circumstances, only detailed laboratory observations can provide estimates of the importance of either of these climatic factors.

The butterfly, *Pieris brassicae* (Linnaeus), widely distributed throughout Europe, southwest Russia, and extreme northwest Africa, reaches Israel at its southern limit in the Near East. There, it can breed only during the winter season, as the summer climate is unfavorable for survival. Bodenheimer (19) presumes that the summer heat is the limiting factor, though in this same area excessively dry winds early in the warm season are known to affect other insect pests unfavorably [Bodenheimer (23)].

The Mexican bean beetle, *Epilachna varivestis,* which originally inhabited the Upper Austral or Transition zone of southern Mexico [Graf (51)], is now distributed in two generally noncontiguous regions, from Central America north into the mountainous western United States, and from southeastern United States north to southeastern Canada. Early studies on the physical ecology of this species appeared to indicate that climate was not a factor limiting its northward spread from Mexico [Hinds (65); Graf (51)], but rather that its limits were set by the geographical range of its food supply [Hopkins (68)]. In its native habitat, however, the beetle is restricted to the cooler, higher elevations of the central plateau of Mexico, delimited generally by the mean annual isotherm of 20°C.; only occasionally is it found in the hotter, damper coastal regions, even though suitable food supplies are available throughout the country [Landis & Plummer (101)]. Laboratory and field observations indicated that in the southwestern United States, where it has been known since the mid-nineteenth century, its move-

ment into western Arizona and southern California is checked by the high temperatures and low humidities of summer, while dispersal eastward into the Great Plains and the western Gulf Coastal areas is checked by high temperatures alone [Sweetman & Fernald (176); Sweetman (174)]. Only when the milder, more humid conditions of the eastern half of the United States are encountered does the beetle appear in injurious proportions again.

Acridid outbreak centers in Siberia can be located by means of average annual temperature isotherms, but, since winter cold is not a limiting factor in acridid ecology, more important climatic indicators of distribution of these centers have been found in mean monthly isotherms for the summer season [Rubtzov (145)]. The pattern of spread of the alfalfa weevil, *Hypera postica* in the Central Valley region of California indicates that it has met a climatic barrier there, presumably hot summer temperatures, since geographical barriers, lack of food supplies, or other obvious factors of climate cannot explain the phenomenon [Michelbacher & Leighly (123)]. This is in contrast to the conclusions of Cook (33), who, on the basis of a bioclimatic analysis of the same species, predicted that the weevil should be able to invade most of California.

Moisture as a limiting factor.—Moisture conditions of the environment, either in the form of water vapor in air or as adsorbed or free water in the soil, ground litter, or plant tissue, act on insects in several different ways, such as by influencing evaporation rates, which in turn modify body water content and body temperature, by affecting behavior and reproduction, or by facilitating pathogenic and parasitic attack [Ludwig (109); Andrewartha & Birch (7)]. Environmental humidity usually becomes a dominant factor affecting insects when temperature conditions are favorable [Uvarov (184)], especially when such humidities are extremely high or low. In many cases, however, the importance of extremes of humidity (dry or wet) is difficult to evaluate, for such conditions are closely correlated with corresponding extremes of temperature.

Nevertheless, in many instances the distributional patterns of insect pests may be closely correlated with conditions of moisture or humidity, regardless of the exact mechanism by which the climatic complex exerts its effect. It has been shown that the lucerne flea, *Smynthurus viridis* (Linnaeus), in Australia is limited to areas with more than 15 inches of rain per year, and that this mean annual isohyet well defines its geographic range [Holdaway (66)]. Because of the seasonal aspect of Australian rainfall patterns, a better measure of the controlling effect of moisture on this pest is the precipitation-evaporation ratio, since whenever this ratio is less than unity, occurrence of the lucerne flea is prevented [Davidson (37)]. Permanent establishment is dependent on the number of successive months with P/E ratios greater than 1.0, together with sufficient temperatures for development of at least one generation.

Similarly, mean annual precipitation levels can be used to define the

geographic distribution of acridid outbreak centers in Siberia (they are restricted to the dry side of the 10-inch annual isohyet), but seasonal precipitation is a more satisfactory indicator delimiting these centers (no mean monthly rainfall during summer greater than 1.2 inches) [Rubtzov (145)].

The precipitation-evaporation ratio has also been used to locate the outbreak centers of the Australian locust *Chortoicetes terminifera* (Walker) and the grasshopper *Austroicetes cruciata* (Saussure) in southern Australia [Andrewartha (5, 6)]. Because of the prolonged egg stage of the latter species (from summer through winter into spring) it was found only necessary to refer to P/E isopleths for the critical spring month when newly hatched nymphs are present. The dry side of the outbreak zone is defined by the P/E isopleth 0.25, while the moist-side (coastward) limits extend to the isopleth 1.0.

In some species, low summer humidities are more important in limiting spread into arid regions than are the excessively high temperatures that also occur in these places at such times. The lady beetle, *Coccinella septempunctata* Linnaeus, widespread in the Euro-Siberian region, protects itself from winter cold by hibernating and from summer heat by estivating. It is found limited in North Africa (north of the Sahara desert), not by heat, but by excessively low humidities [Bodenheimer (22)].

With other species, the most apparent climatic indicator, such as annual rainfall, is in reality an indirect expression of some other aspect of climate, such as seasonal aridity. This may be illustrated by the pattern of distribution of the various species of tsetse flies in tropical Africa. *Glossina morsitans* Westwood, in British West Africa, is found limited in the north in close conformity with the 30-inch annual isohyet. This climatic indicator is, in turn, closely associated with localities having dry seasons of 6 to 7 months' duration, longer dry seasons than this being unfavorable for the species [Nash (126); Jackson (74); see also summary in Andrewartha & Birch (7)].

In many cases, it is found necessary to describe moisture influences on insect distribution as modified by temperature conditions, much as has been done in defining climatic regions on the basis of plant distributions. Rubtzov (144) found the distribution of many species of economically important insects in Russia well defined by lines of equal precipitation-temperature-sum ratios (isopotids). Marcuzzi (116) similarly found that the best climatic indicators of distribution of various tenebrionid beetles in Venezuela were precipitation-temperature ratios, with precipitation-evaporation ratios, mean annual levels of rainfall, evaporation, or temperature being less satisfactory.

In some situations, the dominating effect of precipitation on insect distribution is difficult to assess, although it may be easily demonstrated by other aspects of bioclimatic analysis. For example, the Japanese beetle, *Popillia japonica* Newman, in the eastern half of the United States, must have adequate rainfall during the summer to provide a satisfactory water

content in the soil, and spread into the arid and semiarid western half of the country can be expected to be limited by lack of such summer rains [Fox (47)]. However, the current western limits of the pest do not conform to either the mean annual precipitation patterns in this region of North America nor to similar relative humidity patterns. Hence, on a broad regional basis, suitable soil-moisture conditions cannot always be correlated with the usual meteorological data, and direct bioclimatic correlations fail in such a case. Similarly the distribution of the Mexican bean beetle, *Epilachna varivestis,* cannot be explained on the basis of climatic data alone, since irrigation practices in many local areas of the arid southwestern United States provide the required moisture conditions for its existence there [Sweetman (173)].

An interesting instance of the influence of summer precipitation on insect distribution and abundance is given by the pink bollworm, *Pectinophora gossypiella* (Saunders), in northern India (Punjab). Economically important infestations are limited to those areas where the normally excessively hot temperatures of summer are reduced by the cooling action of midsummer rainfall. Hence summer rainfall is an important climatic indicator for this species, even though the dominating climatic factor is temperature [Khan (77)].

Use of Bioclimatics in Forecasting Distribution

One of the most interesting aspects of bioclimatic analysis is the forecasting of potential distribution of insect species into uninfested areas. Such forecasting attempts are particularly important in cases of foreign insect pests against which quarantine measures have been directed. Bioclimatic analysis formulated with such objectives in mind have been of two general kinds. In one type of analysis the climatic conditions occurring in localities within the known limits of the species are compared with those of uninfested places, and the similarities and differences so found are used as a basis for evaluating the potential invasion of the latter. In the other type of analysis, laboratory studies of the effects of controlled climatic factors on the species are used as the basis for deciding whether climates of uninfested areas are favorable or not. Examples of both methods of analysis are known.

Forecasting by comparison of climates.—Early examples of this type of bioclimatic analysis are afforded by Mansfeld (115), who estimated the potential infestability of Germany by the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), by comparing climatic data of various localities in northern Europe with those of northern United States, and by Cook (33), who estimated the potential distribution of the alfalfa weevil, *Hypera postica,* in northern America by comparing climatographs based on climatic conditions of localities within the original range of the species in Eurasia with similar diagrams based on data from different localities in the United States. A similar approach was used in estimating the potential spread of

the alfalfa weevil in California, although here the analysis was based solely on temperature effects [Michelbacher & Leighly (123)].

With the experience gained by studying the alfalfa weevil, plus similar forecasting techniques applied to the possible spread of the pale western cutworm, *Agrotis orthogonia* Morrison, Cook (34, 35) outlined the procedure involved in comparison of climates as follows: (*a*) collect data on climate in areas of present distribution, (*b*) sort data into damage categories, (*c*) summarize and display data for comparison, (*d*) determine limiting climatic factor(s), (*e*) if possible, check climatic findings with physiological information on species, (*f*) compare results with climates of uninfested areas, and (*g*) construct map showing zones of existing levels of abundance and zones of potential spread. Such a bioclimatic analysis is dependent primarily on only two types of information, climatic data and distributional records.

A subsequent application of this technique was made by Gjullin (50) in the case of the Mediterranean fruit fly, *Ceratitis capitata,* which prior to this time had invaded and then was eradicated from the state of Florida. After searching the literature for information on the physiological effects of the various climatic factors, he concluded that subfreezing temperatures for only relatively short periods were lethal to the fruit fly and that cold, wet winters were a limiting factor in the pupal stage, while dry winters were less damaging. Correlation of distributional records with estimates of damage caused by the fruit fly led Gjullin to conclude that climates like those of Hawaii or South Africa were favorable for the species, that climates characteristic of various places in New South Wales were suitable for medium infestations, and that climates of Spain and France permitted only slight infestations. He presumed that the northern limit of the species in Europe was Paris. The major limiting climatic indicator for economic distribution was considered to be winter temperatures, depending upon the presence or absence of winter rains. By climatographic analysis the United States was zoned according to these limiting factors, as well as for different levels of abundance. Two centers of potentially heavy infestation were considered to exist in southern Texas and southern Florida. Over a much broader zone across the continent, including all of the southern tier of states, occasional outbreaks were considered probable, while periodic establishment was predicted possible within a relatively narrow belt along the northern margin of this zone.

This analysis has been criticized by Quayle (139), who preferred to base estimates of potential spread of this species in the United States on the availability of host fruits and the relative severity of winter, using as a standard for his second criterion the normal winter of Valencia, Spain, where the fruit fly only periodically appears each summer and fall. Quayle concluded that most of Gjullin's zone of possible occurrence would permit severe infestations of the fruit fly to develop, provided suitable food supplies were available. It is interesting to contrast the conclusions of Gjullin

and Quayle with those of Hopkins (68), who, on the basis of his bioclimatic zonation scheme, claimed that Florida would not provide a suitable climatic environment for the Mediterranean fruit fly, because of excessive summer heat and rain.

Other examples of the use of climatographic comparisons in bioclimatic analyses are given by Alfaro (2) for estimating the suitability of the climatic environments of Spain for the Colorado potato beetle, *Leptinotarsa decemlineata,* and by Seamans (153) for outlining the present and potential spread of the wheat stem sawfly, *Cephus cinctus* Norton, in Canada.

Arbuthnot (8) has made an analysis of the widely spread European corn borer, *Pyrausta nubilalis* (Hübner), with particular emphasis on the possible number of generations favored per year. By plotting mean annual temperatures and rainfall totals for various localities within the Old World distribution of this species, limiting conditions for one generation, as well as levels permitting increasing numbers of generations, were determined. With these defining indicators, different localities in the western United States were evaluated for potential establishment of the corn borer. Use of mean annual climatic indicators may overlook certain seasonal aspects of climate and corn-borer bioclimatics. For example, wet springs tend to produce two generations per year, while dry springs tend to favor single generation strains, regardless of spring temperatures or rain in other seasons of the year [Babcock (10)].

Forecasting based on physiological life histories.—Bodenheimer (14) applied this method to the Mediterranean fruit fly, *Ceratitis capitata,* using as a basis the thermal constant and the temperature-development relationship as determined in the laboratory (see above, p. 191). Later the same investigator improved and extended this analysis by including adult sexual development and the period of oviposition in his definition of a generation [Bodenheimer (23)]. By devising a numerical index, termed the bonitation index, for indicating the seasonal (monthly) climatological, phenological, and nutritional suitability of environments, he simplified the comparison of different localities from the standpoint of potential or actual infestability.

The Japanese entomologist Koidsumi and his associates, working on the island of Formosa, utilized the physiological life history approach in bioclimatic analysis directed at an estimate of the possibility of the two tropical fruit flies, *Dacus dorsalis* and *Dacus cucurbitae,* becoming established in Japan. An extensive series of laboratory studies was carried out, primarily based on lethal and favorable effects of temperature, which included determinations of cold-hardiness and ability to acclimatize to winter cold [Koidsumi (78, 79, 83, 84); Shibata (162)], determinations of favorable temperatures for survival and development [Koidsumi (80, 81, 91); Shibata (163, 164)], and the influence of temperature on adult preoviposition development, reproduction, and longevity [Koidsumi (87, 88)]. Most important, besides using the customary constant-temperature-exposure technique for their laboratory analysis, they determined the influences of

alternating temperature conditions on development, reproduction, and survival [Koidsumi (82, 85, 89)]. Following these laboratory studies, artificial temperature environments resembling temperature patterns occurring at various localities in southern Japan were tested for favorability [Koidsumi (86, 89)]. These studies indicated that successful establishment of the two fruit fly species could occur during any month of the year except the winter months of December, January, and February. Previously established infestations would not be able to hibernate or otherwise overwinter in such thermal environments.

Similar studies with these and related fruit fly species, based mainly on the effects of naturally fluctuating temperatures and relative humidities reproduced in specially designed equipment, were made in Hawaii and in Texas in order to estimate the potential establishment and spread of these pests in the United States [Flitters *et al.* (46); Messenger *et al.* (120)]. These analyses based potential establishment on survival over the winter season, duration of winter interruption of breeding, lethal effects of high summer temperatures on adult longevity, and numbers of generations developed per year. A like study of the Mexican fruit fly, *Anastrepha ludens* (Loew), indigenous to northeastern Mexico and normally restricted to Central America as far north as the Rio Grande Valley of south Texas, has shown that, from the climatic standpoint, it is capable of becoming permanently established along the coastal areas of southern California, in southern Texas, along the coastal areas of the Gulf of Mexico, and over most of Florida [Messenger *et al.* (121)]. Dispersal and temporary establishment of this species during favorable seasons were considered possible into the interior regions of southern California, the lower Mississippi River Valley, and along the southeastern seaboard of the country.

Howe and his associates in England carry the bioclimatic analysis a step further by estimating from laboratory studies conditions influencing not only development and survival but also rates of population increase. In the case of the beetle, *Ptinus tectus* Boieldieu, areas of the world were zoned into favorable, doubtful, and unfavorable areas depending on differences and similarities between climatographs of different localities and plots of limiting temperature and humidity combinations for survival and increase [Howe & Burges (70)]. Similar analyses were made for the cigarette beetle, *Lasioderma serricorne* (Fabricius), and the khapra beetle, *Trogoderma granarium* Everts [Howe (69); Howe & Lindgren (71)]. Only where the mean monthly temperatures reach 20°C. or more for at least four months per year can the khapra beetle multiply sufficiently to be an economic problem [see also Hadaway (58)]. When the monthly temperature exceeds this level for six consecutive months, a potentially serious problem will exist. Although the species can tolerate a wide range of humidities [Shulov (167); Hadaway (58)], it cannot compete successfully with other stored-products insects when the mean monthly relative humidity exceeds 50 per cent. These findings support the conclusion that economically impor-

tant infestations of the species can be expected to occur only from southern California to Texas, with serious population levels occurring only in the lower Colorado River area of the southwest, and in south-central Texas. With stored-products pests, the use of heated premises for storage in winter invalidates conclusions based on climatic conditions occurring in exposed, uncontrolled environments.

Forecasting by comparison of species with similar bioclimatic responses.— It is a common technique, especially in plant distribution studies, to correlate the geographic spread of a biotic association or community with certain indicator species [Weaver & Clements (187)]. When the climatic relationships of such an indicator species have been determined, its distribution and abundance can be used as a climatic indicator as well. Gause (49) has discussed this approach for insects, describing the characteristics required of a species in order for it to be an adequate indicator of the environment. This method has been applied in limited degree to economically important species. Sellke (154), finding that the temperature requirements of the oriental fruit moth, *Grapholitha molesta* (Busck), were quite similar to those of the codling moth, *Carpocapsa pomonella* (Linnaeus), predicted that the former may spread throughout the areas in Europe where the latter species has become established. Considering the manner in which *G. molesta* has spread throughout North America, where *C. pomonella* is widely distributed, his prediction may perhaps be quite sound. Nonetheless, Chaudry (28) has shown by laboratory studies that there are small but definite differences in the physiological life histories of these two species. Whether these differences would lead to differences in the respective responses to limiting climatic factors is undetermined. In analyzing the potential spread of *G. molesta* into Pakistan, Chaudry substantiates his bioclimatic conclusions with the presence or absence of *C. pomonella* in the same region.

Perhaps the most intriguing and certainly the simplest approach to the problem of forecasting the potential spread of a pest insect into an uninfested area is that taken by Iwasa & Shibuya (73). In a paper entitled, "Can *Cylas formicarius* propagate under natural climatic conditions in Japan?", they answer their own query by merely introducing the pest into the uninfested environment and observing its ability to grow and multiply therein. While this is certainly a very logical, though unique, approach to bioclimatic analysis, one is apprehensive at the potential quarantine considerations involved in such a scheme.

LITERATURE CITED

1. Adamson, A. M., *Trop. Agr. (Trinidad)*, **18**, 43–47 (1941)
2. Alfaro, A., *Bol. patol. vegetal y entomol. agr.*, **12**, 45–76 (1943)
3. Allee, W. C., Emerson, A. E., Park, O., Park, T., and Schmidt, K. P., *Principles of Animal Ecology* (W. B. Saunders Co., Philadelphia, Pa., 837 pp., 1949)

4. Anderson, D. B., *Ecology*, **17**, 277–82 (1936)
5. Andrewartha, H. G., *Trans. Roy. Soc. S. Australia*, **64**, 76–94 (1940)
6. Andrewartha, H. G., *Trans. Roy. Soc. S. Australia*, **68**, 315–26 (1944)
7. Andrewartha, H. G., and Birch, L. C., *The Distribution and Abundance of Animals* (University of Chicago Press, Chicago, Ill., 782 pp., 1954)
8. Arbuthnot, K. D., *U. S. Dept. Agr. Tech. Bul. No. 987*, 22 pp. (1949)
9. Aubin, M., *Compt. rend. acad. agr. France*, **42**, 553–56 (1956)
10. Babcock, K. W., *Ecology*, **8**, 177–93 (1927)
11. Batzer, H. O., and Benjamin, D. M., *J. Econ. Entomol.*, **47**, 801–3 (1954)
12. Belehradek, J., *Temperature and Living Matter* (Borntraeger, Berlin, Germany, 277 pp., 1935)
13. Blanck, A., *Phytoma*, **5**, 13–17 (1952)
14. Bodenheimer, F. S., *Bull. soc. roy. entomol. Egypt*, 1924, 149–57 (1925)
15. Bodenheimer, F. S., *Z. angew Entomol.*, **12**, 91–122 (1926)
16. Bodenheimer, F. S., *Bull. soc. entomol. France*, 195–98 (1927)
17. Bodenheimer, F. S., *Biol. Zentr.*, **47**, 25–44 (1927)
18. Bodenheimer, F. S., *Z. wiss. Insektbiol.*, **22**, 65–73 (1927)
19. Bodenheimer, F. S., *Biol. Zentr.*, **48**, 714–39 (1928)
20. Bodenheimer, F. S., *Z. vergleich. Physiol.*, **13**, 740–47 (1931)
21. Bodenheimer, F. S., *Problems of Animal Ecology* (Oxford University Press, Oxford, England, 183 pp., 1938)
22. Bodenheimer, F. S., *Bull. soc. Fouad I entomol.*, **27**, 1–28 (1943)
23. Bodenheimer, F. S., *Citrus Entomology* (W. Junk, The Hague, Netherlands, 663 pp., 1951)
24. Bucher, G. E., Cameron, J. W. M., and Wilkes, A., *Can. J. Research*, [D]**26**, 26–56 (1948)
25. Burges, H. D., *Bull. Entomol. Research*, **46**, 813–35 (1956)
26. Buxton, P. A., *Proc. Roy. Entomol. Soc. (London)*, **6**, 27–31 (1931)
27. Chapman, R. N., *Animal Ecology* (McGraw-Hill Book Co., Inc. New York, N. Y., 464 pp., 1931)
28. Chaudry, G., *Bull. Entomol. Research*, **46**, 869–98 (1956)
29. Cloudsley-Thompson, J. L., *Sci. Progr.*, **42**, 46–52 (1954)
30. Cloudsley-Thompson, J. L., *Ann. and Mag. nat. Hist.*, [12]**9**, 305–29 (1956)
31. Cloudsley-Thompson, J. L., *J. Exptl. Biol.*, **33**, 576–82 (1956)
32. Cloudsley-Thompson, J. L., and Sankey, J. H. P., *Ann. and Mag. nat. Hist.*, [12]**10**, 417–24 (1957)
33. Cook, W. C., *J. Agr. Research*, **30**, 479–91 (1925)
34. Cook, W. C., *Ecology*, **10**, 282–93 (1929)
35. Cook, W. C., *Ecology*, **12**, 245–47 (1931)
36. Craighead, F. C., *Insect Enemies of Eastern Forests* (*U. S. Dept. Agr. Misc. Publ. No. 657*, 679 pp., 1950)
37. Davidson, J., *Australian J. Exptl. Biol. Med. Sci.*, **11**, 59–66 (1933)
38. Davidson, J., *J. Australian Inst. Agr. Sci.*, **1**, 105–8 (1935)
39. Davidson, J., *Trans. Roy. Soc. S. Australia*, **60**, 88–92 (1936)
40. Davidson, J., *Australian J. Exptl. Biol. Med. Sci.*, **15**, 113–20 (1937)
41. Davidson, J., *J. Animal Ecol.*, **13**, 26–38 (1944)
42. DeBach, P., Fisher, T. W., and Landi, J., *Ecology*, **36**, 743–53 (1955)
43. Elens, A. A., *Agricultura (Louvain)*, **1**, 19–32 (1953)
44. Elens, A. A., *Agricultura (Louvain)*, **1**, 79–91 (1953)

45. Elens, A. A., *Agricultura (Louvain)*, **1**, 93–100 (1953)
46. Flitters, N. E., and Messenger, P. S., *J. Econ. Entomol.*, **46**, 401–3 (1953)
47. Fox, H., *J. N. Y. Entomol. Soc.*, **47**, 105–23 (1939)
48. Fraenkel, G., and Gunn, D. L., *The Orientation of Animals* (Clarendon Press, Oxford, England, 352 pp., 1940)
49. Gause, G. F., *Ecology*, **11**, 307–25 (1930)
50. Gjullin, C. M., *Ecology*, **12**, 248–58 (1931)
51. Graf, J. E., *J. Econ. Entomol.*, **18**, 116–21 (1925)
52. Graham, S. A., *Ann. Rev. Entomol.*, **1**, 261–80 (1956)
53. Green, G. W., *Can. Entomologist*, **87**, 441–59 (1955)
54. Gressitt, J. L., *Ann. Rev. Entomol.*, **3**, 207–30 (1958)
55. Gunn, D. L., *Z. vergleich. Physiol.*, **20**, 617–25 (1934)
56. Gunn, D. L., *J. Exptl. Biol.*, **12**, 185–90 (1935)
57. Guthrie, F. E., and Decker, G. C., *J. Econ. Entomol.*, **47**, 882–87 (1954)
58. Hadaway, A. B., *Bull. Entomol. Research*, **46**, 781–96 (1956)
59. Harries, F. H., *J. Econ. Entomol.*, **36**, 505–9 (1943)
60. Harries, F. H., *J. Agr. Research*, **69**, 127–36 (1944)
61. Hartshorne, R., *Ann. Assoc. Am. Geographers*, **28**, 165–78 (1938)
62. Haufe, W. O., and Burgess, L., *Ecology*, **37**, 500–19 (1956)
63. Henson, W. R., and Shepard, R. F., *Can. J. Zool.*, **30**, 144–53 (1952)
64. Hesse, R., Allee, W. C., and Schmidt, K. P., *Ecological Animal Geography*, 2nd ed. (John Wiley & Sons, Inc., New York, N.Y., 715 pp., 1951)
65. Hinds, W. E., *J. Econ. Entomol.*, **13**, 486–88 (1920)
66. Holdaway, F. G., *Australia, Commonwealth, Council Sci. Ind. Research, Pam. No. 4*, 23 pp. (1927)
67. Hopkins, A. D., *J. Wash. Acad. Sci.*, **11**, 227–29 (1921)
68. Hopkins, A. D., *U. S. Dept. Agr. Misc. Publ. No. 280*, 188 pp. (1938)
69. Howe, R. W., *Bull. Entomol. Research*, **48**, 9–56 (1957)
70. Howe, R. W., and Burges, H. D., *Bull. Entomol. Research*, **44**, 461–516 (1953)
71. Howe, R. W., and Lindgren, D. L., *J. Econ. Entomol.*, **50**, 374–75 (1957)
72. Hutchinson, A. H., *Ecology*, **21**, 475–87 (1940)
73. Iwasa, T., and Shibuya, T., *Nojikairyo-Shiryo*, **137**, 141–43 (1939)
74. Jackson, C. H. N., *Biol. Revs., Cambridge Phil. Soc.*, **24**, 174–97 (1949)
75. Janisch, E., *Trans. Roy. Entomol. Soc. London*, **80**, 137–68 (1932)
76. Kendeigh, S. C., *Wilson Bull.*, **44**, 129–43 (1932)
77. Khan, M. H., *Indian J. Agr. Sci.*, **8**, 191–214 (1938)
78. Koidsumi, K., *Taihoku Imp. Univ. Entomol. Lab. Contrib. No. 30*, 68 pp. (1931)
79. Koidsumi, K., *J. Soc. Trop. Agr., Taihoku Imp. Univ.*, **4**, 322–59 (1932)
80. Koidsumi, K., *J. Soc. Trop. Agr., Taihoku Imp. Univ.*, **5**, 131–54 (1933)
81. Koidsumi, K., *J. Soc. Trop. Agr., Taihoku Imp. Univ.*, **6**, 395–504 (1934)
82. Koidsumi, K., *J. Soc. Trop. Agr., Taihoku Imp. Univ.*, **6**, 687–96 (1934)
83. Koidsumi, K., *J. Soc. Trop. Agr., Taihoku Imp. Univ.*, **8**, 221–36 (1936)
84. Koidsumi, K., *J. Soc. Trop Agr., Taihoku Imp. Univ.*, **9**, 54–59 (1937)
85. Koidsumi, K., *J. Soc. Trop. Agr., Taihoku Imp. Univ.*, **9**, 411–17 (1937)
86. Koidsumi, K., *J. Soc. Trop. Agr., Taihoku Imp. Univ.*, **11**, 130–51 (1939)
87. Koidsumi, K., *J. Soc. Trop. Agr., Taihoku Imp. Univ.*, **13**, 148–63 (1941)
88. Koidsumi, K., *J. Soc. Trop. Agr., Taihoku Imp. Univ.*, **13**, 229–35 (1941)
89. Koidsumi, K., *J. Soc. Trop. Agr., Taihoku Imp. Univ.*, **13**, 283–97 (1942)

90. Koidsumi, K., *J. Soc. Trop. Agr., Taihoku Imp. Univ.*, **14**, 69–86 (1942)
91. Koidsumi, K., and Kubota, K., *J. Soc. Trop. Agr., Taihoku Imp. Univ.*, **12**, 96–98 (1940)
92. Köppen, W., *Grundriss der Klimakunde* (Walter de Gruyter Co., Berlin, Germany, 388 pp., 1931)
93. Kozhanchikov, I. V., *Z. angew. Entomol.*, **20**, 591–610 (1933)
94. Kozhanchikov, I. V., *Zool. Anz.*, **113**, 7–13 (1936)
95. Kozhanchikov, I. V., *Plant Protect. (U. S. S. R.)*, **16**, 27–34 (1938)
96. Kozhanchikov, I. V., *Bull. Entomol. Research*, **29**, 253–62 (1938)
97. Kozhanchikov, I. V., *Zool. Zhur.*, **17**, 246–59 (1938)
98. Kozhanchikov, I. V., *Proc. Intern. Congr. Entomol., 6th Meeting*, **1**, 59–72 (Madrid, Spain, 1935)
99. Kozhanchikov, I. V., *Compt. rend. acad. sci. U. R. S. S.*, **51**, 241–44 (1946)
100. Kozhanchikov, I. V., *Zool. Zhur.*, **25**, 27–36 (1946)
101. Landis, B. J., and Plummer, C. C., *J. Agr. Research*, **50**, 989–1001 (1935)
102. Larsen, E. B., and Thomsen, M., *Videnskabelige Medd. Dansk naturhist. Foren. Kobenhavn*, **104**, 1–75 (1940)
103. Lathrop, F. H., *J. Agr. Research*, **23**, 969–87 (1923)
104. Leighly, J., *Ecology*, **18**, 180–98 (1937)
105. Lin, S., Hodson, A. C., and Richards, A. G., *Physiol. Zoöl.*, **27**, 287–311 (1954)
106. Lindroth, C. H., *Proc. Intern. Congr. Entomol., 9th Meeting*, **2**, 145–53 (Amsterdam, Netherlands, 1951)
107. Ludwig, D., *Physiol. Zoöl.*, **15**, 48–60 (1942)
108. Ludwig, D., *Physiol. Zoöl.*, **16**, 381–88 (1943)
109. Ludwig, D., *Physiol. Zoöl.*, **18**, 103–35 (1945)
110. Ludwig, C., and Cable, R. M., *Physiol. Zoöl.*, **6**, 493–508 (1933)
111. Lukyanovich, F. K., *Plant Protect. (U. S. S. R.)*, **17**, 15–24 (1938)
112. McPhail, M., and Bliss, C. I., *U. S. Dept. Agr. Circ. No. 255*, 24 pp. (1933)
113. Mail, G. A., *J. Agr. Research*, **41**, 571–91 (1930)
114. Mail, G. A., *J. Econ. Entomol.*, **25**, 1049–53 (1932)
115. Mansfeld, K., *Nachrbl. deut. Pflanzenschutzdienst (Berlin)*, **4**, 45–46 (1924)
116. Marcuzzi, G., *Riv. biol. (Perugia)*, **43**, 399–437 (1951)
117. Martin, C. H., *J. Econ. Entomol.*, **39**, 419 (1946)
118. Martonne, E. de, *Compt. rend.*, **182**, 1395–98 (1926)
119. Merriam, C. H., *U. S. Dept. Agr., Div. Biol. Survey, Bull. No. 10*, 79 pp. (1898)
120. Messenger, P. S., and Flitters, N. E., *J. Econ. Entomol.*, **47**, 756–65 (1954)
121. Messenger, P. S., and Flitters, N. E., *Calif. Avocado Soc. Yearbook*, **41**, 119–27 (1957)
122. Meyer, A., *Chem. Erde*, **2**, 209–347 (1926)
123. Michelbacher, A. E., and Leighly, J., *Hilgardia*, **13**, 101–39 (1940)
124. Mills, H. B., *Yearbook Agr. (U. S. Dept. Agr.)*, 422–29 (1952)
125. Munger, F., and Cressman, A. W., *J. Econ. Entomol.*, **41**, 424–27 (1948)
126. Nash, T. A. M., *Tsetse Flies in British West Africa* (H. M. Stationery Office, London, England, 77 pp., 1948)
127. Noll, J., *Anz. Schädlingskunde*, **18**, 73–78 (1942)
128. Nomura, K., *Sci. Bull. Fac. Agr., Kyushu Univ.*, **13**, 63–66 (1951)
129. Pepper, J. H., *Ecology*, **19**, 565–71 (1938)

130. Pierce, W. D., *J. Agr. Research,* **5,** 1183–91 (1916)
131. Platt, R. B., Collins, C. L., and Witherspoon, J. P., *Ecol. Monographs,* **27,** 303–24 (1957)
132. Powsner, L., *Physiol. Zoöl.,* **8,** 474–520 (1935)
133. Pradhan, S., *Proc. Natl. Inst. Sci. India,* [B]**11,** 74–80 (1945)
134. Pradhan, S., *Proc. Natl. Inst. Sci. India,* [B]**12,** 385–404 (1946)
135. Prebble, M. L., *Can. J. Research,* **19,** 350–62 (1941)
136. Predtetchenskii, S. A., *Rept. Bur. Appl. Entomol., Leningrad,* **3,** 113–99 (1928)
137. Prescott, J. A., *Trans. Roy. Soc. S. Australia,* **58,** 48–61 (1934)
138. Prescott, J. A., *Trans. Roy. Soc. S. Australia,* **60,** 93–95 (1936)
139. Quayle, H. J., *J. Econ. Entomol.,* **24,** 1064–66 (1931)
140. Quilis Perez, M., *Proc. Intern. Congr. Entomol. Proc., 6th Meeting,* **2,** 447–54 (Madrid, Spain, 1935)
141. Roan, C. C., *J. Econ. Entomol.,* **45,** 920–25 (1952)
142. Rubtzov, I. A., *Bull. acad. sci. U. R. S. S.,* 789–824 (1935)
143. Rubtzov, I. A., *Plant Protect. (U. S. S. R.),* **14,** 3–13 (1937)
144. Rubtzov, I. A., *Plant Protect. (U. S. S. R.),* **16,** 3–21 (1938)
145. Rubtzov, I. A., *Problems of Ecological Biocenology (U. S. S. R.),* **4,** 35–88 (1939)
146. Rubtzov, I. A., *Vestnik Zashchity., Rastenii.,* 1941, No. 1, 9–19 (1941)
147. Ryan, F. J., *J. Exptl. Zool.,* **88,** 25–54 (1941)
148. Sacharov, N. L., *Ecology,* **11,** 505–17 (1930)
149. Sanderson, E. D., *U. S. Dept. Agr., Bur. Entomol. Bull. No. 63(1),* 38 pp. (1907)
150. Sanderson, E. D., *J. Econ. Entomol.,* **1,** 245–62 (1908)
151. Sanderson, E. D., *J. Econ. Entomol.,* **3,** 113–40 (1910)
152. Sanderson, E. D., and Peairs, L. M., *New Hampshire Agr. Exp. Sta. Tech. Bull. No. 7,* 125 pp. (1913)
153. Seamans, H. L., *Sci. Agr.,* **25,** 432–57 (1945)
154. Sellke, K., *Nachrbl. deut. Pflanzenschutzdienst (Berlin),* **18,** 69–73 (1938)
155. Shelford, V. E., *Trans. Illinois State Acad. Sci.,* **13,** 257–71 (1920)
156. Shelford, V. E., *J. Econ. Entomol.,* **19,** 251–60 (1926)
157. Shelford, V. E., *Illinois Nat. Hist. Survey, Bull.,* **16,** 307–440 (1927)
158. Shelford, V. E., *Laboratory and Field Ecology* (Williams and Wilkins Co., Baltimore, Md., 608 pp., 1929)
159. Shelford, V. E., *Wilson Bull.,* **44,** 144–57 (1932)
160. Shibata, K., *Trans. Nat. Hort. Soc. Formosa,* **22,** 50–65 (1932)
161. Shibata, K., *Trans. Nat. Hort. Soc. Formosa,* **23,** 335–51 (1933)
162. Shibata, K., *Mem. Fac. Sci. Agr., Taihoku Imp. Univ.,* **26,** 105–16 (1935)
163. Shibata, K., *J. Soc. Trop. Agr., Taihoku Imp. Univ.,* **6,** 95–101 (1936)
164. Shibata, K., *J. Soc. Trop. Agr., Taihoku Imp. Univ.,* **8,** 373–80 (1936)
165. Shibata, K., *J. Soc. Trop. Agr., Taihoku Imp. Univ.,* **13,** 54–62 (1941)
166. Shibata, K., *J. Soc. Trop. Agr., Taihoku Imp. Univ.,* **13,** 113–18 (1941)
167. Shulov, A., *Proc. Indiana Acad. Sci.,* **42,** 1–13 (1955)
168. Smith, A. D., *Ecology,* **21,** 184–91 (1940)
169. Smith, H. S., Essig, E. O., Fawcett, H. S., Peterson, G. M., Quayle, H. J., Smith, R. E., and Tolley, H. R., *Univ. Calif. Agr. Expt. Sta. Bull. No. 553,* 276 pp. (1933)

170. Smith, R. F., *J. Econ. Entomol.*, **47**, 205–10 (1954)
171. Solomon, M. E., and Adamson, B. E., *Bull. Entomol. Research*, **46**, 311–55 (1955)
172. Stellwaag, F., *Anz. Schädlingskunde*, **16**, 109–13 (1940)
173. Sweetman, H. L., *Ecology*, **10**, 228–44 (1929)
174. Sweetman, H. L., *Ann. Entomol. Soc. Am.*, **25**, 224–40 (1932)
175. Sweetman, H. L., *J. Econ. Entomol.*, **26**, 320–5 (1933)
176. Sweetman, H. L., and Fernald, H. T., *Mass. Agr. Expt. Sta. Bull. No. 261*, 32 pp. (1930)
177. Sweetman, H. L., and Wedemeyer, J., *Ecology*, **14**, 46–60 (1933)
178. Talbot, M., *Ecology*, **15**, 416–39 (1934)
179. Tehon, L. R., *Illinois Nat. Hist. Survey, Bull.*, **17**, 321–46 (1928)
180. Teterovskaya, T. O., *Parazitologicheskii Sbornik, Leningrad*, **11**, 262–77 (1949)
181. Thornthwaite, C. W., *Geograph. Rev.*, **21**, 633–55 (1931)
182. Thornthwaite, C. W., *Ecology*, **21**, 17–28 (1940)
183. Trewartha, G. T., *An Introduction to Climate*, 3rd ed. (McGraw-Hill, New York, N.Y., 402 pp., 1954)
184. Uvarov, B. P., *Trans. Roy. Entomol. Soc. (London)*, **79**, 1–247 (1931)
185. Uvarov, B. P., *Ecology*, **8**, 309–11 (1932)
186. Uvarov, B. P., *Quart. J. Roy. Meteorol. Soc.*, **71**, 226–27 (1945)
187. Weaver, J. E., and Clements, F. G., *Plant Ecology* (McGraw-Hill Book Co., New York, N.Y., 601 pp., 1938)
188. Wellington, W. G., *Can. Entomologist*, **80**, 56–82 (1948)
189. Wellington, W. G., *Sci. Agr.*, **29**, 201–15 (1949)
190. Wellington, W. G., *Sci. Agr.*, **29**, 216–29 (1949)
191. Wellington, W. G., *Trans. Roy. Soc. Can.*, [3]**5**, 89–101 (1950)
192. Wellington, W. G., *Sci. Agr.*, **30**, 209–34 (1950)
193. Wellington, W. G., *Meteorol. Monographs*, **2**, 11–18 (1954)
194. Wellington, W. G., *Ann. Rev. Entomol.*, **2**, 143–62 (1957)
195. Wellington, W. G., Sullivan, C. R., and Green, G. W., *Can. J. Zool.*, **29**, 339–51 (1951)
196. West, A. S., Jr., *Ann. Entomol. Soc. Am.*, **29**, 438–48 (1936)
197. Williams, C. B., *Quart. J. Roy. Meteorol. Soc.*, **71**, 222 (1945)
198. Williams, C. B., *Nature*, **157**, 214–15 (1946)

ETHOLOGICAL STUDIES OF INSECT BEHAVIOR[1]

By G. P. Baerends
Zoological Laboratory, University of Groningen, Netherlands

Introduction

The term "ethology" has come into use in European zoology to distinguish the objective study of animal behavior from a more subjective animal psychology. A great advance was made when particularly Lorenz and Tinbergen, on the basis of their observations and experiments, formulated a number of hypotheses which opened the way to more systematic analytical studies. The great value of these concepts is that they have given ethology a method of thinking, and have led to duly considered experiments. When more workers, often educated in other fields, became interested in ethology, criticism against several of these concepts arose (104). This development is fortunate because it will never destroy but can only improve the theoretical background of the study of behavior. For more information on these concepts I refer to Tinbergen (166) and Baerends (10, 11).

In this article I shall use ethological concepts as a framework to review data on the behavior of insects. It will become clear that the system of concepts, mainly derived from work on vertebrates, is suitable for this. This does not mean that the hypothetical mechanisms are thought to be homologous; they are analogous, just like the respiratory or digestive systems of several widely different taxonomical groups. Because of space restrictions, I had to leave out all purely descriptive papers, however valuable they are; the data reviewed I have grouped in three sections: Function, Causation, and Comparison and Evolution.

Function

Experimental research into the function of behavioral elements is relatively scarce and is mainly concentrated on behavior involved in the communication between animals of the same and of different species.

Daanje (36) has described in great detail the behavior by which leaf-rolling beetles [*Apoderus coryli* (Linnaeus) and *Attelabus nitens* (Scopoli)] construct the tubes in which they deposit their eggs. By comparing normal tubes and failures he is able to give functional interpretations of the different activities. The cut made in the leaf serves to reduce the turgor sufficiently to make rolling possible, but, because of the developing egg, it must not reduce the leaf's moisture too much. Consequently, the method by which thick leaves and thin leaves are cut is different.

[1] The survey of the literature pertaining to this survey was completed in February, 1958.

A few authors have tested the survival value of defensive mechanisms that make use of cryptic behavior in connection with procryptic coloration or of protective displays. De Ruiter (46) has proved experimentally the protective effect of the morphological and behavioral characteristics of some kinds of stick caterpillars and several species of countershaded caterpillars (48) against such visual predators as chaffinches and jays. The anatomical substrates as well as the orienting mechanisms warranting maximal protection from countershading have developed convergently in different species. The protection from resemblance to sticks drops markedly as soon as a bird has found a stick caterpillar by accident, usually caused by a moment of inappropriate behavior. However, as a result of this conditioning, the bird is directed not only toward caterpillars but also toward sticks. Therefore, the reward is frequently not forthcoming, and the original situation is restored.

After de Ruiter (47) had shown that the sudden display of the red, eyespot-bearing hindwings in the moth *Smerinthus ocellatus* (Linnaeus), when attacked, protects the animal from being eaten by chaffinches, Blest (17) made a more general experimental study of the effects of eyespot patterns on birds (chaffinches, yellow buntings and great tits), using living butterflies as well as models. The sudden presentation of circular patterns, particularly if shaded to appear three-dimensional, was proved to be more effective in releasing escape responses in the birds than noncircular patterns of the same size. A group of concentric circles of the same area as a single circle surpasses the latter in releasing value. Besides this intimidation effect, a deflection effect was shown by presenting mealworms artificially ornamented with small eyelike spots. Such spots direct the attack and can, if they are appropriately situated, deflect it from more vulnerable parts of the animal, to give it a possibility for escape. The threat function of stridulation in scorpions was proved by Alexander (4).

Overt fighting occurs in insects, as in vertebrates, mainly between animals of the same species. The fighting usually serves to maintain minimum interindividual distances even in more or less gregarious insects [the digger wasp *Ammophila campestris* Latreille (8), dynastid beetles (14), dragonflies (123)]. Only a few cases have been reported in which the behavior of these insects resembles territory defense in the vertebrates. The males of the damselfly *Calopteryx* defend, mainly against males of their own species, territories that decrease in size with an increase in population. They do not keep to their territories for more than one day. Reproductively motivated males of solitary bees (174) and bumble bees (74, 91) perform regular flights along definite routes, attacking other bees (irrespective of species) and trying to mate with conspecific females. Honey bees attack dark, moving objects on the routes between hive and foraging area (102) and on the foraging area if competition rises above a critical level (187). Defense of the colony (28, 64, 68, 99) is the task of special guards that, after being alarmed by the infiltration of bees from

other colonies, start inspecting bees at the hive entrance. Intruders other than robbers are allowed to pass if they behave dominantly when intercepted. Submissive bees are extensively examined and often mauled. Bees trying to escape frequently are killed. The guards can distinguish robbers by their typical swaying flight, which is in itself a response to the congestion of bees at the entrance of the hive caused by the examination procedure.

Different functions have been suggested for the often elaborate behavior patterns introducing copulation, viz., (*a*) selection of a partner of the right species, right sex, and right physiological condition, (*b*) stimulation of the partners, (*c*) inhibition of motivations (aggression, flight) incompatible with the consummatory sexual act, (*d*) attraction and direction of a partner, and (*e*) rebuffing of competitors.

In the Orthoptera, courtship has been extensively studied in *Gryllus* (80, 183, 195) and in grasshoppers (84, 86). In *Gryllus* and in the Acridinae courtship consists of a progressive exchange of stimuli between male and female. The response of one partner to a stimulus given by the other releases the next response in the latter, and so on. In this way, courting acts as a selection mechanism. Among the stimuli given, song plays an important part (69), and different types of song may be distinguished. The normal song is produced by a single male; it passes into a rival song if a competitor is met, but into a courtship song if a female is encountered. In some species a female willing to copulate answers the male song (83, 130), and the attraction song develops (*Werbewechselgesang*). In addition, songs may occur in some other stages of the courtship. Most of the songs are highly specific. Weih (189) showed that males of three related *Chorthippus* species occurring in the same area respond with rival song in only a minority of cases to males of one of the other species. Perdeck (130) found that in the sympatric species *Chorthippus brunneus* Thunberg and *Chorthippus biguttulus* (Linnaeus) the difference in song is the only barrier to hybridization. Both male and female answered exclusively to the sound of conspecific partners. Moreover, Perdeck could prove that the songs orient both sexes toward each other, increase the locomotion and the number of copulation attempts of the male and the willingness of the female.

Courtship in the Odonata (23, 24, 25) and Lepidoptera (35, 112, 167) consists of a similar system of exchange of stimuli between male and female. At least in the beginning of courtship, these stimuli are mainly visual. Remarkable are the differences between races of *Calopteryx splendens* (Harris), and also—even within one race—the differences between populations [Buchholtz (24)].

In Collembola (120), in *Lepisma saccharina* Linnaeus (164), and in scorpions (7), courtship has the effect of directing the female toward a spermatophore which is meanwhile deposited on the substrate. During this performance the male of *Euscorpius italicus* Herbst stings the female re-

peatedly; the at first very aggressive nature of this stinging dies down gradually. The inhibition of aggression in the female is a function of the display of the male salticid spider, in which it waves its forelegs (29 to 33, 55).

Aggregations in insects can be caused passively, by the attraction for every individual of special local conditions [mosquito swarms: (54, 125)] or actively, by the attractiveness of conspecific individuals [bees: (68, 87, 101)]. The latter case affords a possibility for communication, even in insects that do not live in real colonies like *Periplaneta* (57). Communication in social insects is a most fascinating field, too extensive to be dealt with properly in this review. With recent work on Isoptera the names of Emerson (58), Grassé (71, 72, 73), and Schmidt (148, 149, 150) are connected; with work on ants those of Le Masne (105), Schneirla (151 to 157), and Vowles (184); and with honey bees those of Butler (27, 28), Free and co-workers (64 to 68), von Frisch and co-workers (176 to 182), Lindauer (107 to 110), and Ribbands (144).

In the interrelations between members of the colony special movements with antennae, legs, and mouth parts play a very important role, often in combination with the exchange of substances such as food and caste-determining secretions (21, 111, 185). Schneirla (156) proposes to extend the use of the old term "trophallaxis" to all these specific stimulations that arouse or increase action or serve to facilitate physiological processes in the colony. Free (66) studied the factors influencing the direction in which food is passed between two workers of a honey-bee colony; the age of the workers and the age difference between them, the quantity of food contained in the honey stomach, and previous experience all play a role. Allen (2, 3) found the worker bees more interested in a stationary queen than in one laying eggs or moving about on the comb. Moreover, this interest decreased as the swarming urge developed. Le Masne (105), who made in ants the as yet most detailed study of this trophallactic behavior, reports that special movements of the worker, which can be imitated by the experimenter, release the production of substances in the larvae. By certain movements of the head, the larva can accept or refuse food offered, and both the ant worker and the human experimenter must make special movements in order to get the food accepted. Observations that larvae are sometimes aggressive against the workers suggest that these movements may have an appeasing function, analogous to that observed in so many vertebrates. Free (65), studying food exchange in worker honey bees, found that movements of the head are important in offering food, movements of the antennae in begging for it. An important factor in keeping the group together is the colony-specific scent, which is carried in the fur of the bees, not in the only species-specific scent gland [Renner (143)]. Deleurance (37 to 42) and Pardi (128, 129) have studied the system and the consequences of the interrelations between the members of *Polistes* colonies. The community is started by a fertilized female,

later on assisted by other females developed from the queen's eggs. Between these females a rank order exists, maintained chiefly by signal movements and regulating the distribution of liquid foods through trophallaxis. Pardi is of the opinion that this rank order is supported by differences in the development of the ovaries caused by differences in the food received. Deleurance, however, gives arguments against this view; he found normal reproductive behavior in castrates and, in special experiments, egg laying in submissive females.

In the beginning of the season the brood develops normally and produces sound imagines [Deleurance (41, 42)]. In autumn, however, many of the larvae perish, and newly emerged imagines are often abnormal. This brood abortion even leads to destruction of the colony. The abortive phase is not primarily caused by a shortage of workers, but by the workers' becoming old and less capable of feeding the larvae thoroughly and with the right kind of oligodynamic secretions. Moreover, microbe infections break out as a result of incomplete nutrition and insufficient care by the workers. The young queens emerge at the end of the summer, hibernate and go through a long diapause, which must lead to cyclic periodicity in the wasps.

In the social insects, communication plays an important role in increasing the efficiency and co-ordination of foraging (88). Ants are brought into an excitatory state by stimuli from larvae, callows, and other workers, particularly workers coming home with booty. In this condition the ants will leave the nest and follow the scented trails to the food sources. [Vowles (184); Sudd (165)]. The flying honey bees have developed a much more elaborate behavior pattern for communicating the discovery of a food source; we will deal with that on page 225f.

Causation

Efferent systems.—For a thorough ethological description of behavior, one must choose units that are neither too complex nor too simple. Although a description in terms of muscular contractions and relaxations would be most exact and objective, this is practicable only in profound studies of details. The activities distinguished usually involve the entire body of the insect, or at least a considerable part of it. For them Lorenz introduced the term "instinctive activities" and raised the question whether they are physiologically distinct from other units of behavior. Before trying to answer this question for the Arthropods, I shall first discuss the features thought by Lorenz to characterize these "instinctive activities" and consider whether they are present in insect behavior.

First, Lorenz stressed the specific stereotypy, to be demonstrated in the last section, on page 226f. Secondly, he thought that the instinctive activity was only released (or initiated) by external stimuli but maintained and concluded by internal mechanisms independent of external stimulation. This idea was based upon the observations of threshold fluctuations under

constant external stimulation and on the impossibility of stopping an activity like egg retrieving in the graylag goose by taking away the object after the movement had started.

In arthropods several cases have been described that are similar to Lorenz's classical example. The digger wasp *Ammophila,* once having started the movement of dragging its caterpillar backward into the burrow, continues this action until it has almost disappeared into the nest entrance, even if the prey is carefully taken away (8). In *Musca* (78) the stereotyped cleaning movements of the legs are still performed after amputation of the extremity to be cleaned; this is true as well as of mutants with abnormal wings that cannot be touched adequately by the normal movement. In *Gryllus* cleaning of the antennae is carried out by the maxillae, toward which they are moved by the frontal legs. Severing the connectives behind the subesophagial ganglion interferes with the co-ordination between the three elements. The antennae are brought downward, but they are not picked up by the legs and brought toward the mouth parts, which nevertheless perform the cleaning movements [Huber (80)]. All these examples show that the co-ordinations of these patterns are brought about by the central nervous system in great independence of feed-back stimuli from the object. Extirpation of the sense-organ-carrying tarsi in *Musca* [Heinz (78)] did not influence the cleaning movement of the operated leg, suggesting that the central co-ordinating mechanisms are also relatively independent of at least these sensory organs on the leg. That stimuli from these organs are important for the initiation of the movement follows from the fact that cleaning movements occur less frequently in operated legs. The importance of the sensory organs can be shown further by bringing objects like a leg or a wing toward a leg performing cleaning movements *in vacuo;* then the movements direct themselves appropriately with respect to these objects. This observation supports another conclusion from the egg-retrieving experiments: the instinctive activity is composed of two parts, a mainly centrally co-ordinated fixed pattern, relatively independent of external stimulation (*Erbkoordination*), and a steering component (taxis) continuously influenced by external stimuli and adjusting the former to the environment. Mittelstaedt's work on the snapping activity in mantids (122) is an example of an analysis of this steering component.

The work of Peters (131 to 134, 136) and Mayer (119) has shown that different elements of the orb webs of spiders bear mathematical relations to each other. The authors have suggested that the relationship could be established by stereotyped motor patterns on which taxis components are superposed. Case building in caddis-fly larvae is another instance of the same principle [Brickenstein (22)].

Work on insects renders considerable support to the concept of a hierarchy of instinctive behavior (Tinbergen, 166). According to this idea, instinctive activities are grouped in physiological systems ("instincts") characterized by the fact that all behavioral elements belonging to it share

common causal factors. The various "subsystems" may again be grouped under systems of higher order. In many cases each system serves one specific function. A system is usually activated by a special stimulus situation inside or outside the animal; the role of autonomous impulse production has often been suggested, but has never been conclusively proved. The first example in insect behavior has come from the progressive provisioning behavior of *Ammophila campestris* Latreille [Baerends (8)]. This digger wasp cares simultaneously for different nests. Provisioning takes place in three phases. The first consists of digging the nest and storing the first caterpillar, on which the egg is deposited. In the second phase one or two other caterpillars are added, and in the last three to seven. At the end of the third phase the nest is closed permanently. The phases of care for one nest are intercalated between those for another. The wasp always starts the second and third phase by paying a visit without bringing a caterpillar. This visit was experimentally proved to function as an inspection. Provisioning follows only if an enclosed larva is present, and the number of caterpillars is determined by the size of the larva and by the amount of food present at the inspection visit. However—and this is the most important point in this context—the behavior of the wasp can be influenced only at an inspection visit, not at all by experimental changes before a provisioning visit. After the last provisioning visit, the wasp will conclude the third phase by a permanent closure, even if, just before this visit, the whole content is taken from the cell. This means that during the inspection visit a system comprising and controlling the entire provisioning behavior of a phase is activated. What brings the activated state to an end is unknown, but it certainly need not be a new external stimulus. Recently Tsuneki (168, 169) has discovered another digger wasp practicing progressive provisioning and rearing simultaneously more than one larva: *Bembix nipponica* Smith. Whereas *A. campestris* makes separate single-cell nests for every larva, *B. nipponica* extends its compound nest with new cells. Tsuneki has experimentally demonstrated that, as in *Ammophila,* provisioning in *Bembix* is adjusted to the developmental degree of the larva and to the amount of food in the cell. Here, too, the wasp can cease her work without any additional external stimuli after having stored a "pre-determined" amount of food. Although Tsuneki does not distinguish between inspecting visits and provisioning visits, this difference may also exist in *Bembix,* for not in all experiments did these wasps adjust their behavior to changes of the cell contents; in several identical trials they failed to respond. Tsuneki does not report successive phases in the provisioning of a nest, but the inconsistent results of experiments in which he offered more than one larva in different artificial cells could be explained by a maladjustment of the experiments to such phases. These are much easier to detect in *Ammophila,* because all cells have their separate very short burrows and leave the greater part of the activity of the wasp open to direct observation.

A second example may be derived from Deleurance's work on nest build-

ing in *Polistes* (43, 44, 45). Building activity is started by the stimulus situation: absence of empty cells in the nest. It is spread over the day in bouts, each composed of the three different types of building: pedicel construction, the formation of a new cell, and the heightening of the cell walls, performed in this succession. Between the building bouts other behavior phases are shown. The total amount of building per bout decreases exponentially during the day (pedicel building more rapidly than cell formation); so does the frequency of building during the time each single bout lasts. Some internal extinction process seems, therefore, to bring a building bout or an entire building phase to an end. No influence of external factors was found; the number of cells built and the number of eggs laid during a day are not closely adjusted to each other. Although the presence of the ovarium is essential for continued nest building, a close correlation between building activity and details in the development of the ovaries does not exist (38, 39). A larva is a powerful stimulus to nest building; and it particularly releases heightening of the cell walls. Larvae may even reactivate ovariectomized females to resume building for the time of their presence in unsealed cells (42).

In contrast to the above-mentioned examples, cases have been reported in which an external situation determines the end of a phase. This stimulus may simultaneously release the following phase of a chain.

In an experimental study of web building, König (90) found that the fixed motor patterns used are subordinated to three different systems, phases in the building of an orb web, viz.: (*a*) building radii, (*b*) attaching the temporary spiral, (*c*) attaching the viscid spirals. The end of each phase is determined by external (consummatory) stimuli and initiates at the same time the next phase. Phase *a* can be prolonged or shortened experimentally by removing or adding radii respectively. The role of internal factors is shown by a reduction of the building rate when the removal of radii is continued for a long time. Phase *b* ends when the spider has reached the marginal thread of the web, phase *c* when the central part is reached. Web repairing belongs to a separate behavioral system.

Witt (192, 193) showed that various drugs interfere with web building in specifically different ways. No interpretation of these actions can yet be given. However, this method seems promising, provided it is combined with careful analytical behavior studies.

Huber (79, 80) managed to induce different systems of instinctive activities in *Gryllus* by puncturing different parts of the brain (mushroom bodies and central body). In this way he could produce separately the common song, the rival song, and the courtship song with the appropriate behavior patterns. It appears that by these lesions he removed causal inhibitory factors specific to each of these systems, evidently at a relatively high level; for the lower motor center for chirping is situated in the second thoracic ganglion. The male is able to perform the initial courtship only if nervous stimuli, reaching the brain from the last abdominal ganglion, report the

presence of a spermatophore. If this is not present, or if the nervous connection has been severed, the male shows only after-courtship. Huber has recently started experiments to stimulate the *Gryllus* brain electrically (126).

The great importance of stimuli from outside the central nervous system in regulating complicated behavior sequences has been shown in the following investigations.

Van der Kloot & Williams (171, 172, 173) studied cocoon construction in the silkworm *Platysamia cecropia* (Linnaeus). Spinning is induced by internal stimulation related to the presence of silk glands, but it can be hastened or postponed by the external situation. Stimuli from the substrate and gravity direct the spinning activities. Two different and incompatible motor patterns are alternately performed. The periodical switch-over from one pattern to the other, for which the animal turns around in the cocoon, is not induced by external factors, but the presence of silk glands is necessary for this turning and for the regular performance of the spinning patterns, although blocking of the spinnerets does not interfere with it. [Wiedbrauck (191) found in *Gallionella* that touching will induce spinning in caterpillars without silk glands.] The cocoon consists of two envelopes. The spinning of the inner envelope is started when 60 to 70 per cent of the silk has been spun. Experimental interference with cocoon construction showed that this happens irrespective of the results of the spinning activity. It was proved to depend entirely on the amount of silk passed through the spinneret. Surgical procedures showed that the mechanisms integrating cocoon construction are localized in the brain.

The reproductive behavior of the grasshopper, *Euthystira brachyptera* Ocskay, is characterized by a succession of phases in which the female accepts the male actively, tolerates it passively, or refuses it, copulation being prevented while the eggs are laid. By studying the order in which these elements succeed each other in normal, virgin, and castrated females, Renner (141, 142) found that the beginning of refusal, which precedes egg laying, is closely correlated with the time the eggs leave the ovarioles and pass into the oviduct. Active readiness to accept the male occurs only when the female has no sperm in the receptaculum seminis; otherwise the readiness is passive. After one mating, several batches of eggs can be deposited at intervals, in which only passive readiness occurs. The phase of prevented copulation lasts as long as there is a spermatophore inside the female. In normal females this spermatophore is gradually dissolved in the receptaculum; in castrates it remains and repulses the male when the latter touches it with its penis.

Schneirla (156, 157) studied in the field and in the laboratory the regulation by external stimuli of the cyclic changes in behavior of doryline ants. In accord with the large size of the colonies and the carnivorous way of life, periods of statary colony life in a well-sheltered place alternate with nomadic phases characterized by great raiding activity and temporary nests

or bivouacs. The stimuli regulating the alternation of the phases come from the brood. The appearance of callows initiates the nomadic phase; the presence of larvae maintains it until this generation passes into the pupa stage. Then the statary life is resumed, during which the queen produces a new batch of eggs. The emergence of the sexual brood initiates a whole complex of behavior leading to colony division (153, 154, 155).

The influence of hormones on behavior was demonstrated by Piepho (139) and Wiedbrauck (191), who by implanting corpora allata in caterpillars of *Galleria mellonella* (Linnaeus) and *Bombyx mori* (Linnaeus) could postpone the larval phase, increase the number of instars, and also make intermediates between larva and pupa. Different behavior patterns serve the construction of webs for casting the larval skin in passing from one instar to the next and of cocoons for pupation. If the experimental animal is an intermediate, both types of spinning behavior occur in combination, resulting in an intermediately shaped cocoon. Therefore, the spinning behavior is, like the ontogenetical development, directly or indirectly influenced by the equilibrium between metamorphose and corpora allata hormones.

The proper way to detect and distinguish different behavioral systems (instincts) is by statistical and experimental analysis, not by a merely logical classification according to functions, as has been done by Tsuneki (169) and Evans (61) for *Bombix* species.

In the moth *Automeris aurantiaca* Weymer, Bastock & Blest (12) carried through such a quantitative analysis for the activities occurring when a resting moth is disturbed: display, flight, neutral posture, walking, and settling accompanied by a rocking movement. These activities can occur in sequences, but of all orders theoretically possible only ten have been observed. Each series begins with display and ends with settling and rocking. The amount of rocking is greater the rarer or the earlier in the sequence flight occurs. The frequency of rocking when it occurs just after eclosion is directly correlated with neutral posture, but inversely with flight in sequences produced later in life. Neutral posture is inversely correlated with flight, flight inversely with display. After a discussion of several alternative explanations, the authors interpret these and similar facts by postulating two different systems, one responsible for settling, the other for flying. Each system exerts inhibiting forces on the other. Rocking is thought to be linked with the settling system; there is evidence that neutral posture results from a simultaneous activation of both systems.

If a system comprises several instinctive activities, what determines which of these is performed at a given moment? The work of Bastock & Manning (13) on the courtship of *Drosophila* is concerned with this problem. Three elements are distinguished: orientation, vibration, and licking, which do not replace, but are superposed upon, one another. Although there is a tendency to begin orientation and then add successively vibration and licking, this order is by no means fixed; any one element may follow another. As the components are not released by special responses from the fe-

male, the hypothesis was tested that the elements receive common excitation from the same center but have different thresholds. Arguments in support of this explanation are that for vibration plus licking the average bout length is inversely correlated with the length of orientation bouts, that the percentage of licking increases with the bout length of vibration, and that the length of the vibration bouts increases with the total time spent courting a female. In addition to this mechanism, the course of the courtship is controlled by inhibitory stimuli produced by the female.

In both papers just reviewed evidence was given for the possibility of interaction between two incompatible mechanisms or systems. We know from the work on vertebrates that such conditions usually lead to ambivalent and displacement behavior. In *Drosophila* cleaning behavior occurs when the courtship is simultaneously aroused and inhibited. The authors interpret this as displacement activity and suggest that elements of courtship like wing vibration may originate from it.

Not many examples of displacement activities in insects have been described. Kullenberg (93) mentions wing vibration in a male digger wasp copulating with an orchid flower. Huber (80) reports that males which are no longer able to respond with singing, because the connections between the brain and the thoracal ganglia have been cut, perform cleaning and eating movements after touching the female with the antennae. Crane (34) considers the defensive display of mantids to have originated from a conflict between the tendencies to stay motionless and to flee, through ritualization of ambivalent as well as displaced activities. She suggests similar possibilities to explain the display in salticid spiders (32).

We have seen that the instinctive activities and the superimposed systems are usually activated by external stimuli. The intensity of the reaction depends on the excitatory state of the system and on the strength of the external stimulus. A higher intensity is generally characterized by a longer duration of the activity and by participation of a greater number of muscle groups (80).

This is nicely illustrated in experiments carried out by Schwinck (160) on the response of males of *Bombyx mori* (Linnaeus) to the scent of the females. The response consists of a sequence of several components that replace one another or are added in a fixed order. Dilution of the scent or reduction of the receptor capacity through amputation leads to incomplete responses. The greater the dilution or the part amputated, the earlier in the fixed chain the response is broken off and the longer it takes for the moth to pass from one link to the next.

Just as in vertebrates, the strength of the external stimulation and the degree of excitation of the system at the moment the animal is externally stimulated act in combination and can support each other. Buchholtz (23) found *Calopteryx* males that for a long time had not been visited by a female much more responsive to models than males that had regularly courted females or models in their territories.

In Lorenz's original concept the excitatory state was thought to be caused primarily by autonomous production of action potential. In the discussion above we have met with many examples showing the influence of various kinds of internal stimulation on the excitatory state. Moreover, as Kennedy (89) has argued, a qualitative change of activity independent of external or peripheral internal stimuli does not necessarily imply spontaneous impulse production of motor centers. He compares the alternation of flight and plant feeding in aphids with the phenomena of "successive induction" and "rebound" described by Sherrington in vertebrates. Matthes (118) claims to have found an example of an autonomous activity in the turning the psychid moth *Fumea crassiorella* Bruand performs just before metamorphosis, after having attached its case to a tree. His argument is that no external stimuli release or direct turning; however, he did not take into account the influence of internal stimuli (like, for instance, hormones). The fact that evidence in favor of autonomous impulse production is very weak in vertebrates and, as far as I know, is totally lacking for arthropods does not necessarily mean that such production does not exist. But we do know that numerous other possible ways of influencing the central excitatory state exist. How difficult it may be to detect these influences is well illustrated by Weis-Fogh's finding that the flight in *Schistocerca gregaria* Forskål, which can be initiated in different ways, is maintained by feed-back stimuli from wind-sensitive hairs on the head (190). It will be fruitful to further research to realize the importance of feed-back stimuli and, in general, of excitatory and inhibitory stimulation of systems in the animal. At the present state of our knowledge a distinction between reflex and instinctive mechanisms on the basis of the autonomous character of the latter, as was first suggested by Lorenz, is no longer justified. It seems to me that the differences are not of a qualitative character but rather correspond to differences in level of integration (10, 11).

In Lorenz's earlier concept, the excitatory state of the efferent mechanisms was thought to fluctuate but the afferent systems were considered to be constant. This idea cannot be maintained; Dethier (49, 52) demonstrated peripheral and central adaptation in the sensory mechanism for taste of *Phormia regina* (Meigen). This means that at the present state of our knowledge we have to realize that various mechanisms, on the way from sense organ to motor unit, can be responsible for variations in the reaction to a constant stimulus situation.

Precht (140) and Drees (55) have developed a very interesting method of spotting these mechanisms and of investigating causal factors they have in common. In feeding, courting, fleeing, and spinning behavior of salticid spiders they have studied quantitatively the waning of a response at repeated stimulation, the influence of it on other responses, the recovery of a response, and the interaction of other responses with this recovery. Moreover, they have tried the influence of deprivation of food or of a sexual partner on the system. Groups of activities were shown to have common

causal factors; members of the same group usually differ in the rate at which they bring down the level of the excitatory state of a group. Different groups may depend on common sources of excitation. Unfortunately, the interpretation of the authors is very much influenced by the use of "psycho-hydraulic" models. Although it cannot be denied that these models have stimulated and suggested the experiments, in my opinion a more operational treatment of the data obtained would be desirable.

Only very little research has been done on the ontogeny of motor patterns. Petersen *et al.* (137), working with imagines of *Pieris napi* (Linnaeus) and *Pieris bryoniae* Ochsenheimer, studied the increase in height of phototropic flights released under standard conditions in series of butterflies that got more, less, or no flight experience during the first five days. The result at the fifth day is the same in all series, showing that the influence of experience is negligible. Evidence is given that the gradual increase in the height reached and in the starting angle in the experienced series can be attributed to a gradual hardening of the wing cuticula. Mayer (120) found that spiders prevented from practicing irregular provisional spinning in the youth were not handicapped when building their first real orb web, Bastock & Blest (12) found the behavior patterns of flight already present in moths taken from the cocoon. Therefore, in these three cases, no influence of conditioning on the development of motor patterns was found.

Afferent mechanisms.—The preceding paragraph cites evidence that behavioral elements of different degrees of complexity can be released, maintained, terminated, inhibited, primed, and directed by specific external situations. The same object may serve several effects, each often through different physical properties of the object. For instance, Laarman (97) found that the host-seeking behavior of *Anopheles maculipennis atroparvus* Thiel can be released by CO_2 and directed by differences in temperature and by moisture. The odor of blood has both effects.

An object may release a complex of behavioral elements that are superimposed on or substituted for one another. In such cases we must realize that each element may receive its own releasing and directing stimuli from the object. Examples are the successive stages in the hunting behavior of *Notonecta* (9) and in the host-finding behavior of the chalcid wasp *Mormoniella vitripennis* (Walker), a parasite of muscoid pupae (56). After activation of a system, further stimulation determines which of several subordinate alternatives will be released. Once hunting is activated in a salticid spider, a rapid motion of a simple model at a distance of 1 to 10 cm. releases a running approach, but a slow movement of a three-dimensional object at a distance of 4 cm. elicits creeping (55).

Experimental investigations into the part played by different physical properties of a stimulus situation in releasing or directing a definite response have been carried out in different groups of insects. The first study along this line was carried out in Lepidoptera by Tinbergen *et al.* (167) on the stimuli eliciting the first action in the courtship of the male grayling,

Eumenis semele (Linnaeus), that is, the sexual pursuit flight. Various cardboard models were offered to the males. Models with original wings have no greater value than simple plain paper models, irrespective of color, showing that wing pattern and color are unimportant for this reaction. Characters of shape were of little importance, but size, distance and, above all, the type of movement were proved to be essential.

Petersen *et al.* (138) repeated these experiments with *Pieris napi* and *Pieris bryoniae*. All males of both species have a marked preference for white models without the black markings, which is remarkable as some females of *P. napi* and all of *P. bryoniae* are yellow. Though hybrids can be produced, they are very rare in nature. Consequently, there must be some barrier that particularly prevents *bryoniae* males from mating *napi* females; it is suggested that differences in scent act as such.

According to Crane (35), in *Heliconius erato hydara* Hewitson yellow has the highest value for releasing feeding, but orange-red for sexual responses. Roosting companions, however, are searched for by scent, not by color. Ilse (82) found in *Pieris brassicae* (Linnaeus) a preference for red, yellow, blue, and violet when feeding, and for green when depositing eggs. Stride (163) studied the courtship-releasing factors in *Hypolimnus misippus* (Linnaeus). The males of this species have black wings, with a white patch in the center; the females, mimetics of *Danaus chrysippus* (Linnaeus), occur in different forms, all having brown wings. Black wings on models inhibit sexual responses in the male; the effect of hindwings is stronger than that of forewings. White on the hindwings has a very strong inhibitory effect. Consequently, the male is not normally attracted to the female of *D. chrysippus alcippus* (Cramer), which closely resembles the female of *H. misippus* but has white hindwings. It is suggested that the inhibitory effect on the male of a white hindwing is the ultimate reason why no *H. misippus* female form mimetic to *D. chrysippus alcippus* could develop.

Magnus (113, 114), studying the same reaction in *Argynnis paphia* with a well-designed merry-go-round-like apparatus, found color (Orange, Ostwald 4 pa) and movement to be of great importance. Females of the black valesinaform have less value for releasing the pursuit flight in all males. A plain orange model supersedes in value a model bearing the natural wing pattern. To satisfy the need for movement, a rotating cylinder bearing orange and black bands suffices, and its releasing value increases with the speed of rotation.

Lederer (103) states that *Limenites* males search visually for females but by scent for food. However, learned visual cues help it return to already known food sources.

Schwinck, studying sexual attraction in bombycid moths, found the scents were not typical for the species (158, 161). Only at short distances can the scent direct the moth; at greater distances it activates and maintains searching behavior which can be then directed by air currents (159).

In Odonata, Buchholtz investigated with models the factors releasing

sexual pursuit flight in *Calopteryx* (23, 24) and *Platycnemis* (25). In *Calopteryx* only one wing suffices for the full response; it has to be yellowish blue or yellowish green and, in addition, must be highly transparent (60 to 80 per cent). The shape is of no importance; the optimal size is about the normal one. The female wings are always less transparent than those of the male. Some forms of *C. splendens* use dark wing tips as a specific character. In contrast to *Calopteryx,* models attracting a *Platycnemis* male need at least a wing, a head, and a thorax. The marking pattern on the thorax and even more that on the prothorax have a relatively high releasing value. The head is of importance primarily through shape and size. Here markings may easily be omitted, although they can to some extent compensate a wiping out of the thorax markings.

In Hymenoptera, several authors (Von Frisch, Knoll, Hertz, etc.) have contributed to our knowledge of the features of flowers that attract bees. A survey of this work has been given by Kugler (92). Characters of high value are certain colors, size, disruptedness, three-dimensionality, velvety surfaces, marking contrasts, and scents (9). Lecomte (98, 100) studied the stimulus situation releasing attack in honey bees, and found movement, contour, and dark color important. The value of the model is considerably enhanced when poison glands of the bees are added to it and increases with the number of glands applied. It looks here as if the releasing mechanism combines stimuli of different sensory organs—an argument for its central situation.

In Coleoptera, Beebe (15) found that rain releases the entire reproductive instinct in the Hercules beetle *Megasoma elephas* (Fabricius).

In a descriptive study of the songs in Orthoptera, Faber (62) demonstrated numerous differences that exist between species and within species between songs of various function. As mentioned under "Function," these songs play an important role in sexual isolation and stimulation. Several authors have tried, with all kinds of model experiments using flutes, comb wheels, the human mouth, and electronic apparatus, to detect the elements in the song essential for response in grasshoppers or crickets [Weih (189), Busnel *et al.* (26), Haskell (76)]. Most important is the pulse structure which results from details in the way and manner in the rhythm of the movements of the stridulatory organs. The properties of the receptor are still insufficiently known; it seems to be particularly sensitive to differences in amplitude (81). The specificity varies from species to species; even in related forms, one species may react to a wide range of different songs [*Chorthippus biguttulus* (Linnaeus)], whereas another [*Chorthippus brunneus* (Thunberg)] is very selective [Weih (189)].

Using the salticid spider *Epiblemum scenicum* (Clerck), Drees (55) made a profound study of the characters of the external stimulus situation releasing feeding and sexual behavior. In both cases the characters used are visual, at least until close contact with the object is made. Most important for eliciting feeding activities are size, movement, three-dimensional-

ity, contrast against the background, and disruptedness. Very valuable characters in releasing sexual responses are the legs of the object. In models these are maximally effective when they show the right thickness in relation to the size of the body and when the relative distances between the different "joints" of each leg match the natural ones. Other important characters are the breaking up of the body into cephalothorax and abdomen, the relative proportion between those parts, and the black and white stripe pattern on the abdomen. Cephalothorax and abdomen are able to elicit a response only when combined with legs or with the stripe pattern. The more valuable factors added to the model, the more components of the courtship are performed. A two-dimensional object releases only waving the forelegs, a three-dimensional body with two-dimensional legs evokes also the zigzag dance. Crane (32) obtained similar pictures from a less detailed but more comparative study of this group. She finds, however, that in some species scents of the female release sexual responses. In the threatening display of *Corythalia xanthopa* Crane, the yellow clypeus has a strong releasing value, while a white sub-basal abdominal band in the female has directive properties.

Plant-eating insects are usually chemically attracted by one or few of the many substances present in the plant. It stands to reason that these substances are more specific in oligophagous than in polyphagous insects (Dethier, 50).

From these experiments we may draw the following general conclusions. Very often when an insect reacts to an object, only a few of all physical properties the animal is able to perceive actually contribute to releasing the response. Properties that are of importance in evoking one special reaction are often of negligible value for another response. For instance, *Eumenis* does not discriminate between colors in the sexual pursuit flight, but is attracted by blue and yellow when visiting flowers. *Heliconius* (35) and *Pieris* (82) are attracted by different colors when feeding than when the sexual or the parental instincts are activated. In some other examples we have seen insects with well-developed visual and olfactory senses responding to optical stimuli for one activity and to scent for another. The same phenomenon has been found in vertebrates (9, 10, 11, 16). It leads to the hypothesis that between the receptors and the motor centers there are intercalated other afferent mechanisms, probably of a more central character, that evaluate with regard to a special activity the information passed by the sensory organ. This mechanism or group of mechanisms was called by Lorenz the "releasing mechanism." The relative value a character has for releasing an activity is not absolutely fixed but can vary within limits in accordance with quantitative aspects of these characters. Bright objects have less value in eliciting the sexual flight in *Eumenis* than darker ones: the darker the model, the stronger the releasing effect. Whereas brown is the normal color of the female, black releases a stronger response. Likewise, *Argynnis* males react most strongly to models

with the greatest surface of the preferred orange color, even if the increase of this surface was brought about at the cost of the typical black marking pattern, which was proved to be of negligible value. Weih (189), when imitating the song of *Chorthippus* with the mouth, could rule out real grasshoppers. This means that a feature can artificially be made supernormal.

Schneirla (155) criticized the hypothesis of a centrally situated mechanism for the valuation of stimuli, arguing that the possibility that "preferences" are caused by special properties of the sensory organ was overlooked. He particularly favored the idea of attributing the high value of disrupted patterns in so many insects to the great sensitivity of the insect eye for flicker. It will be wise to realize that the principle of Schneirla's critique may be justified in several cases. But some cases of preference for disrupted patterns are known that do not seem to fit this explanation. Buchholtz (23, 24) found the marking pattern of the thorax, prothorax, and head in *Calopteryx* each of very different value to the male. Vogel (175), analyzing the situation releasing the sexual jump in the male of *Musca domestica* Linnaeus, found plain black models to be of higher value than models disrupted in the center, and of lower value than models with peripheral disruption. Sakagami (145, 146, 147) has tested Schneirla's critique of the original interpretation of Herz's experiments, by training bees to choose between homogeneous figures on a disrupted background. He has made great effort to form an idea of how the disrupted pattern is received by the eye of a flying insect. Schneirla's explanation does not fit the fact that characters playing a role in one reaction are of no effect in another. Neither does it account for the observation that relations between parts of the releasing object are of considerable importance (e.g., proportions of leg and body parts in salticid spiders, as mentioned before).

After evaluation, the releasing mechanism has to combine the information of the different stimuli. Experiments in which one kind of active stimulation is replaced by another show that only the ultimate combined value of all stimuli counts, no matter what features are contributing. A salticid spider jumps on a two-dimensional model only when it moves, but on a three-dimensional also when it stands still. Only when the internal readiness to act is very low do all stimuli have to be present to elicit the reaction. At lower threshold, however, the stimuli prove to be interchangeable.

The releasing mechanism was formerly thought to be constant. Prechtl (10, 11) has shown for vertebrates that it can become adapted; Dethier's experiments make it likely that the same is true in insects (49, 52). Moreover, the releasing value of an object is influenced by the motivational state of the animal: Drees (55) found that the optimal size for a model releasing a feeding response increases with hunger.

Ethologists have for many years spoken of *innate* releasing mechanisms. In fact, some observations and experiments show that the properties of the releasing mechanism may be present without any relevant experience.

Crane's experiments with *Heliconius,* for instance, gave the same result in carefully reared inexperienced butterflies as in experienced ones (35). Drees (55) found no difference between experienced and inexperienced salticid spiders in regard to the stimuli releasing courtship. Kugler (92) reports several cases of spontaneous color preference in Hymenoptera, Lepidoptera, and Diptera. Inexperienced *Limenites* react to the scent of blackberry flowers.

On the other hand, some evidence can be produced that already the first reaction of an animal to an object is influenced by experience, e.g., in the response of plant-eating insects to their host plants (50, 51). Many more examples are known of a modification of the releasing mechanism by learning processes. Bumble bees visiting hound's-tongue learn the general form of the plant, those frequenting foxglove become conditioned to the color of the flowers [Manning (116)].

Very interesting are two cases in which a gradual quantitative change in host preference was found after several generations had been reared on a less preferred host. When Laarman (97) reared the man-parasitizing *Anopheles maculipennis atroparvus* consistently on rabbits he obtained an increased tendency to parasitize the latter host. Finlayson (63) found similar changes when rearing the bethylid *Cephalonomia waterstoni* Gahan on four species of *Laemophloeus* differing in host value. However, in none of these experiments was the preference completely reversed. It is suggested that the alternative host species differ in the strength of the same attractive agent and that in times of shortage of hosts the less preferred are also parasitized.

That complicated learning processes naturally occur in insects is particularly clear from experiments on orientation in Hymenoptera (166, 186). There is ample evidence that the type of the characters learned is predetermined, partly, of course, by limitation corresponding with the capacity of the sense organs, but also by the features of more centrally situated mechanisms. Otherwise it is difficult to understnd, for instance, that of the very similar and related digger wasps *Philanthus* and *Bembix* the first prefers three-dimensional objects as beacons to learn the situation of their nests, while the second prefers large, flat areas [Van Iersel (170)].

Social releasers.—As Lorenz has pointed out, the occurrence of releasing mechanisms reacting to definite characters in an object together with the capacities of the sense organs must in the course of evolution lead to a further specialization of these characters in the object, if this is an organism benefiting from the response. Many morphological and behavioral structures that might have evolved this way can be suggested. Only a few cases in insects have been studied to some extent.

The survival value of eyespots in defensive display has already been mentioned on page 208. Eyespots occur in a great many insects, particularly Lepidoptera; the morphological basis for their development is present in the general color pattern of the Rhopalocera [Baerends (9), Blest (17)]. Flowers show several characteristics that may be considered as adaptations,

partly to some preferences present in a great many insects, partly to those of a special group. Honey guides that are not only optically (115) but also olfactorily (106) discernible to the insects are a further appeal to the releasing mechanisms (whether inborn or acquired). Such guides not only attract but also direct—only visually (115), not olfactorily (20). Flower constancy increases the possibilities for evolutionary developments (117). Most remarkable is the case of flowers of some orchids (*Ophrys, Cryptostylis*) that imitate the females of definite wasp species and are consequently fertilized by the males trying to copulate. These flowers may be said to parasitize (9) on a releasing mechanism present in the male insects. The *Ophrys* case has been extensively studied by Kullenberg (93 to 96), who showed that the flower of *Ophrys muscifera* Hudson has developed visual and tactile structures attracting the males of the digger wasp species *Gorytes mystaceus* (Linnaeus) (color pattern, thick hairy labellum, antennaelike petals) and a sexually attractive scent very similar to the odor of the female. Other instances of chemical social releasers are the so-called ectohormones that in social insects prevent the development of egg-laying females other than the queen (16, 27, 111, 127; see also pp. 39 to 58).

The best known case of a behavioral social releaser (and social "director") is the dancing of the honey bees (182). Information about distance and direction of food sources (177) and new dwelling places (107, 109) can be given this way. The indications account for wind effects (181), detours (176), and steep sections in the route, although a real indication of height differences is not possible (179). The distance is best indicated by the duration of tail wagging in one single run (180). The concentration of the food is not indicated by a special element in the dance but by total duration and vivacity and by the offering of food samples. Weather and time of the day influence the dance, particularly when food is obtained at a great distance (19, 180). Use of the sun, directly [even if it is behind the clouds (178)] or indirectly through the polarization of the blue sky, and an inner clock (142) enable the bees to indicate and to find the right direction of a food source at night (110). The bees have to learn the sun's arc typical of a geographical position (109) but have an inherited knowledge of the direction of the movement of the sun: clockwise on the northern, counterclockwise on the southern hemisphere (88a).

Different "dialects" have been found in different races of the honey bee (77, 121). Round dance and wagtail dance occur in all four species of *Apis*—in *Apis florae* Fabricius in a more primitive form (horizontally on the comb without transposition of gravity for light). A dance that only informs other workers of the presence, not of the direction and distance, of the food was found in the more primitive *Trigona iridipennis* Smith. (108, 109). Speculations on the origin of the dances have been made [Haldane & Spurway (75)], but the most important contribution in this respect was Dethier's discovery that the fly *Phormia regina* (Meigen), after having encountered a drop of sugar, starts a kinetic moving about which becomes more regular with crowding of the flies, which can be di-

rected by light and gravity, and which is in intensity and duration influenced by the concentration of the sugar and the time lapse between withdrawal of stimulation and onset of the response (53). Experiments by Wittekindt (194) show that the form of the dances is not acquired by experience and, moreover, may suggest that the different types of dance correspond to different levels of an excitatory state.

Comparative Studies and the Evolution of Behavior

The stereotypic character of insect behavior has invited many authors to search for homologies between instinctive activities of different species and to compare the differences and similarities between behavior patterns of more and less related taxonomical groups.

Heinz (78) gives detailed descriptions of the cleaning behavior of *Musca domestica* Linnaeus and *Sarcophaga caranaria* Linnaeus and compares these with cleaning behavior in other groups of Diptera. He particularly considers the manner of cleaning of the second pair of legs, the cleaning of the antennae, the readiness to clean, and the "elegance" of the preening movement. The Brachycera, Nematocera, and Pupipara are in many ways different. Cleaning activities are most frequent in the Brachycera; the long-legged Nematocera have a much lower readiness to clean. In general one may say that the similarity of cleaning movements in different groups increases with closer relationship. Exceptions to this rule may usually be correlated with exceptional body structures.

The cleaning movements bear relation to the localization of sense organs as well as to special life habits of the insect. Weidmann (188) develops a system of some *Drosophila* species, using as a criterion for relation the number of courting activities species share. His result is identical with a classification based on morphological criteria. Crane (29 to 33) compares 15 species of salticid spiders and groups them according to degree of dependence on chemical stimuli and to the display and manner of locomotion. Runners have chemotaxis and distant chemoperception, lack intermale display and are morphologically characterized by a low carapax and few spines; hoppers rarely use chemical stimuli, have distinct threat and courtship displays, a high carapax, and numerous spines. A group with an intermediate type of locomotion uses distant chemoreception, has slightly differentiated displays but shows frequent fighting; carapax and spines have intermediate characters. Therefore, in many regards, morphological and ethological characters go together. In her comparative study of the defensive behavior of 15 mantid species, Crane (34) distinguishes four types: camouflage, flight, startle display, and attack. All species use camouflage and flight (dodging, jumping, or dropping). Six species have a defensive display which consists of facing the threat with tegmina and wings raised, while the other parts of the body, particularly the forelegs and abdomen, are similarly exhibited. Conspicuous markings are demonstrated. Four species possess an active attack consisting of striking with the forelegs.

The morphologically primitive groups show unspecialized defensive behavior; display occurs in two specialized groups.

Peters (135) has compared web building in tropical spiders. Jacobs (85) reviews comparative studies of antennae cleaning in Rhynchota (Pössinger), turning behavior in Coleoptera (Fürsch) and the mode of fixation of the pupa girdle in Rhopalocera (Söllner). Jacobs (84, 86) himself has made an extensive study on grasshoppers, comparing the customary classification with the occurrence of different types of courtship. In the *Podisma* and *Tetrix* types the male simply jumps at the visually detected female, in the latter type sometimes after having made a special movement. In the *Caliptamus* and *Oedipoda* types the male when approaching to jump produces a "jumping song"; in *Oedipoda* this is produced with the mandibles only. The *bicolor, stigmaticus,* and *maculatus* types are all characterized by a real song in addition to the jumping song. In the first type this is the "common song," in the other two the "courting song," and it is particularly developed in the latter type. Similarity in behavior and in morphological features were shown to correspond very well in large as well as in small taxonomical groups.

Ethological arguments can not only confirm a classification along morphological lines; they sometimes make classification possible where morphology has not yet found suitable criteria. Considerable differences in behavior between different populations of *Ammophila campestris* (differences in prey, season, method of closing the nest, scheme of progressive provisioning) led to the splitting up of this species in two new ones (1). Evans (59) managed to distinguish two morphologically very similar *Anoplius* species with the help of ethological characters. Crane (30) used morphological and ethological characters to distinguish three species of the jumping spider genus *Corythalia.* Fulton (70) and Alexander (5) found song variations to be the best cue to differentiation of species of the field cricket genus *Acheta.* Emerson (58) and Schmidt (148, 149), in their studies of termites, use the nests as species-specific examples of "frozen behavior" to characterize the species and to study phylogeny in this group. According to Schmidt (150), the nests tell more about the phylogeny of the species than do the morphological characters of the termites. Emerson suggests the names "ethospecies" and "ethotype" for cases in which ethological differences are predominant.

Comparative studies open the possibility of developing hypotheses on the evolution of behavior. Particularly in the digger wasps interesting progress has been made with regard to parental behavior. Evans (59) suggests that in the Pompilidae it has developed from bethyloid-scolioid ancestors possessing the behavior elements of hunting, oviposition on a prey, paralyzing, and prey transporting. It is thought that using an already existing nest place is a more primitive pattern to which first the behavior of closing the nest and later on also that of digging a nest have been added. Types that dig a nest after they have secured a prey (the most

general pattern in the pompilids) are considered to be more primitive than those that dig a nest before setting out on a hunting trip [*Pompilus plumbeus* Fabricius and *Priocnemis exaltatus* (Fabricius); the latter is one of the few species in this group that builds a compound nest]. In recent years a considerable amount of work along these lines has been done on the evolution of provisioning behavior in the Bembicini [Nielsen (124), Tsuneki (168, 169), Evans (61)]. This group is particularly interesting because progressive provisioning is practiced which in *Bembix nipponica* Smith and *Bembix brullei* Guérin even developed to simultaneous care for more than one larva. For several species the first prey, on which the egg is deposited, functions chiefly as a pedestal. This seems to be the origin of other species' habit of laying the eggs in the empty cell, after arranging sand grains in such a way that the egg remains upright when glued to them. It is interesting to compare this trend with a convergent evolution in the Stizini which culminates in the practice of preparing, by gluing together sand grains, a small mound of earth on top of which the egg is laid at a 45° angle [Evans (60)].

Blest (18) discusses the evolutionary development of protective display in Saturnioidea and Sphingidae. The five types of display (a generalized escape behavior, rhythmic display, sustained static display, mixed display, and vestigial display) do not correspond to taxonomic divisions but to the type of coloration of the moth. Sustained static displays are correlated with either mimetic or eyespot patterns, mixed displays (which consist of rhythmic and static compounds) with eyespot patterns, and vestigial displays with a reduction or replacement of patterns. Generalized escape behavior is combined with generalized cryptic coloration, cryptic behavior with specialized procryptic coloration. It is suggested that the displays have evolved from preparatory flight movements, performed by moths which are too cold to fly, in this order: rhythmic displays, mixed displays, sustained static displays; each stage may have led independently to vestigial displays.

Alexander (5, 6) shows how basic pulse rate reproductively isolates amongst themselves three morphologically related crickets (*Nemobius carolinus* Blatchley, *Nemobius confusus* Blatchley and *Nemobius melodius* Blatchley) which are—by a rhythmical element they have in common—distinguished from the morphologically different *Nemobius maculatus* Blatchley. The evolution of feeding preferences in phytophagous insects has been discussed by Dethier (51).

In this context we have finally to consider what is known about the genetics of insect behavior. Von Hörmann (183) crossed the *Gryllus* species *campestris* and *bimaculatus*. Fighting, which occurs much more frequently in *campestris* than in *bimaculatus*, is dominant in F_1; the results of backcrossing suggest monofactorial transmission. The same is true for certain trembling movements of the antennae. In *campestris*, just before the courtship song starts, the elythrae are raised once, in *bimaculatus* re-

peatedly and in combination with sound production (precourtship song). The F_1 hybrids are either intermediate (producing one single sound) or behave like one of the parents. In this case also backcrosses indicate monofactorial transmission.

Spieth (162), studying mating behavior in 14 strains of the *Drosophila viridis* group, found only quantitative differences between them. Sexual isolation is mainly the result of interaction of courtship discrimination (chiefly practiced by the males) and sexual drive (important in both sexes). A definite balance between both components has adaptive value in ensuring a maximal number of successful copulations. With regard to cleaning behavior, Heinz (78) found no (qualitative) differences between various *Drosophila* mutants, even not when organs or parts of them had dropped out.

LITERATURE CITED

1. Adriaanse, A., *Behaviour*, **1**, 1–34 (1947)
2. Allen, M. D., *Brit. J. Animal Behaviour*, **4**, 14–22 (1956)
3. Allen, M. D., *Brit. J. Animal Behaviour*, **5**, 81–84 (1957)
4. Alexander, A. J., *Behaviour*, **12**, 339–52 (1958)
5. Alexander, R. D., *Ann. Entomol. Soc. Am.*, **50**, 584–602 (1957)
6. Alexander, R. D., *Ohio J. Sci.*, **57**, 153–63 (1957)
7. Angermann, H., *Z. Tierpsychol.*, **14**, 276–301 (1957)
8. Baerends, G. P., *Tijdschr. Entomol.*, **84**, 68–275 (1941)
9. Baerends, G. P., *Symposia Soc. Exptl. Biol.*, **4**, 337–60 (1950)
10. Baerends, G. P., *Handbuch der Zool.*, **10**(3), 1–32 (1956)
11. Baerends, G P., *Acta Physiol. et Pharmacol. Neerl.* **7**, 42–75 (1958)
12. Bastock, M., and Blest, A. D., *Behaviour*, **12**, 243–84 (1958)
13. Bastock, M., and Manning, A. *Behaviour*, **7**, 85–111 (1955)
14. Beebe, W., *Zoologica*, **32**, 109–16 (1947)
15. Beebe, W., *Zoologica*, **29**, 53–58 (1944)
16. Bier, K. H., *Ins. Soc.*, **1**, 7–19 (1954)
17. Blest, A. D., *Behaviour*, **11**, 209–56 (1957)
18. Blest, A. D., *Behaviour*, **11**, 257–309 (1957)
19. Boch, R., *Z. vergleich. Physiol.*, **38**, 136–67 (1956)
20. Bolwig, N., *Brit. J. Animal Behavior*, **2**, 81–83 (1954)
21. Brian, M. V., *Ann. Rev. Entomol.*, **2**, 107–20 (1958)
22. Brickenstein, C., *Abhand. bayer. Akad. Wiss., Math. Naturw. Kl.*, **69**, 1–44 (1955)
23. Buchholtz, C., *Z. Tierpsychol.*, **8**, 274–93 (1951)
24. Buchholtz, C., *Z. Tierpsychol.*, **12**, 364–86 (1955)
25. Buchholtz, C., *Z. Tierpsychol.*, **13**, 13–25 (1956)
26. Busnel, R., Dumortier, B., and Busnel, M., *Bull. biol. France et Belg.*, **90**, 219–86 (1956)
27. Butler, C. G., *Proc. Roy. Soc. (London)*, [B]**147**, 275–88 ((1957)
28. Butler, C. G., and Free, J. B., *Behaviour*, **4**, 262–92 (1952)
29. Crane, J., *Zoologica*, **33**, 1–38 (1948)
30. Crane, J., *Zoologica*, **33**, 139–45 (1948)
31. Crane, J., *Zoologica*, **34**, 31–52 (1949)
32. Crane, J., *Zoologica*, **34**, 159–214 (1949)
33. Crane, J., *Zoologica*, **35**, 253–61 (1950)
34. Crane, J., *Zoologica*, **37**, 259–93 (1952)
35. Crane, J., *Zoologica*, **40**, 167–95 (1955)
36. Daanje, A., *Behaviour*, **11**, 85–155 (1957)
37. Deleurance, E. P., *Compt. rend.*, **223**, 871–72 (1946)
38. Deleurance, E. P., *Compt. rend.*, **226**, 514–16 (1948)
39. Deleurance, E. P., *Compt. rend.*, **227**, 866–67 (1948)
40. Deleurance, E. P., *Compt. rend.*, **230**, 782–84 (1950)
41. Deleurance, E. P., *Behaviour*, **4**, 104–15 (1952)
42. Deleurance, E. P., *Ins. Soc.*, **2**, 285–302 (1955)
43. Deleurance, E. P., *L'instinct dans le comportement des animaux et de l'homme*, 105–50 (Masson & Cie., Paris, France, 796 pp., 1956)
44. Deleurance, E. P., *Ann. sci. nat. Zool. et biol. animale*, 11, 91–222 (1957)
45. Deleurance, E. P., *Behaviour*, **11**, 67–84 (1957)

46. de Ruiter, L., *Behaviour*, **4**, 222–32 (1952)
47. de Ruiter, L., *Levende Nat.*, **55**, 121–26 (1952)
48. de Ruiter, L., *Arch. néerl. zool.*, **11**, 1–57 (1955)
49. Dethier, V. G., *Biol. Bull.*, **103**, 178–89 (1952)
50. Dethier, V. G., *Trans. Intern. Congr. Entomol., 9th Meeting (Amsterdam, Netherlands, 1951)*, **2**, 82–88 (1953)
51. Dethier, V. G., *Evolution*, **8**, 33–54 (1954)
52. Dethier, V. G., *Quart. Rev. Biol.*, **30**, 348–71 (1955)
53. Dethier, V. G., *Science*, **125**, 331–36 (1957)
54. Downes, J. A., *Trans. Roy. Entomol. Soc. (London)*, **106**, 213–36 (1955)
55. Drees, O., *Z. Tierpsychol.*, **9**, 169–207 (1952)
56. Edwards, R. L., *Behaviour*, **7**, 88–112 (1954)
57. Ehrlich, H., *Z. Tierpsychol.*, **5**, 497–552 (1943)
58. Emerson, A. E., *Am. Museum Novitates*, **1771**, 1–31 (1956)
59. Evans, H. E., *System. Zool.*, **2**, 155–72 (1953)
60. Evans, H. E., *Behaviour*, **7**, 287–303 (1955)
61. Evans, H. E., *Comparative Ethology of Digger Wasps of the Genus Bembix*, (Comstock Publishing Associates, Ithaca, N. Y., 248 pp., 1957)
62. Faber, A., *Mitt. staatl. Museum Naturk., Stuttgart*, **287**, 1–198 (1953)
63. Finlayson, L. H., *Behaviour*, **2**, 275–316 (1950)
64. Free, J. B., *Behaviour*, **7**, 233–40 (1954)
65. Free, J. B., *Brit. J. Animal Behaviour*, **4**, 94–101 (1956)
66. Free, J. B., *Brit. J. Animal Behaviour*, **5**, 41–49 (1957)
67. Free, J. B., *Behaviour*, **12**, 233–42 (1958)
68. Free, J. B., and Butler, C. G., *Behaviour*, **7**, 305–16 (1955)
69. Frings, H., and Frings, M., *Ann. Rev. Entomol.*, **3**, 87–106 (1958)
70. Fulton, B. B., *Evolution*, **6**, 283–95 (1952)
71. Grassé, P. P., *Colloq. intern. centre natl. recherche sci. (Paris)*, **34**, 7–17 (1952)
72. Grassé, P. P., *Colloq. intern. centre natl. recherche sci. (Paris)*, **34**, 323–31 (1952)
73. Grassé, P. P., *L'instinct dans le comportement des animaux et de l'homme*, 561–75 (Masson & Cie., Paris, France, 796 pp., 1956)
74. Haas, A., *Z. vergleich. Physiol.*, **31**, 281–307 (1949)
75. Haldane, J. B. S., and Spurway, H., *Ins. Soc.*, **1**, 247–83 (1954)
76. Haskell, P. T., *Brit. J. Animal Behaviour*, **5**, 139–48 (1957)
77. Hein, G., *Experientia*, **6**, 1–6 (1950)
78. Heinz, H. J., *Z. Tierpsychol.*, **6**, 330–71 (1948)
79. Huber, F., *Verhandl. deutsch. zool. Ges. (Freiburg)*, 138–49 (1952)
80. Huber, F., *Z. Tierpsychol.*, **12**, 12–48 (1955)
81. Huber, F., *Naturwissenschaften*, **43**, 317–21 (1956)
82. Ilse, D., *Nature*, **140**, 544–45 (1937)
83. Jacobs, W., *Verhandl. deutsch. zool. Ges. (Mainz)*, 238–44 (1949)
84. Jacobs, W., *Z. Tierpsychol.*, **2**, 169–216 (1950)
85. Jacobs, W., *Verhandl. deutsch. zool. Ges. (Freiburg)*, 115–38 (1952)
86. Jacobs, W., *Z. Tierpsychol.*, Suppl. 1, 1–228 (1953)
87. Kalmus, H., *Brit. J. Animal Behaviour*, **2**, 63–71 (1954)
88. Kalmus, H., *Brit. J. Animal Behaviour*, **2**, 136–39 (1954)
88a. Kalmus, H., *J. Exptl. Biol.*, **33**, 554–65 (1956)
89. Kennedy, J. S., *Discovery*, **17**, 311–12 (1956)

90. König, M., *Z. Tierpsychol.*, **8**, 462–92 (1951)
91. Krüger, E., *Z. Tierpsychol.*, **8**, 61–75 (1951)
92. Kugler, H., *Einführung in die Blütenökologie*, 93–127 (Gustav Fisher Verlag, Jena, Germany, 278 pp., 1955)
93. Kullenberg, B., *Oikos*, **2**, 1–19 (1950)
94. Kullenberg, B., *Oikos*, **3**, 53–70 (1951)
95. Kullenberg, B., *Svensk Botan. Tidskr.*, **50**, 25–46 (1956)
96. Kullenberg, B., *Zool. Bidr. Uppsala*, **31**, 254–354 (1956)
97. Laarman, J. J., *The Host-Seeking Behaviour of the Malaria Mosquito* (Doctoral thesis, Univ. of Leiden, Leiden, The Netherlands, 144 pp., 1955)
98. Lecomte, J., *Behaviour*, **4**, 60–66 (1951)
99. Lecomte, J., *Apiculteur (Sect. Sci.)*, **97**, 1–5 (November 1953)
100. Lecomte, J., *Ins. Soc.*, **1**, 49–57 (1954)
101. Lecomte, J., *Ins. Soc.*, **3**, 195–98 (1956)
102. Lecomte, J., *Z. Tierpsychol.*, **13**, 26–30 (1956)
103. Lederer, G., *Z. Tierpsychol.*, **8**, 41–61 (1951)
104. Lehrman, D. S., *Quart. Rev. Biol.*, **28**, 337–63 (1953)
105. Le Masne, G., *Ann. sci. nat. Zool. et biol. animale*, **11**, 1–56 (1953)
106. Lex, T., *Z. vergleich. Physiol.*, **36**, 212–34 (1954)
107. Lindauer, M., *Z. vergleich. Physiol.*, **37**, 263–324 (1955)
108. Lindauer, M., *Z. vergleich. Physiol.*, **38**, 521–57 (1956)
109. Lindauer, M., *Bee World*, **38**, 3–39 (1957)
110. Lindauer, M., *Naturwissenschaften*, **44**, 1–6 (1957)
111. Lüscher, M., *Ins. Soc.*, **3**, 119–28 (1958)
112. Magnus, D., *Z. Tierpsychol.*, **7**, 436–49 (1949)
113. Magnus, D., *Verhandl. deut. zool. Ges. (Tübingen)*, 317–25 (1954)
114. Magnus, D., *Deut. entomol. Tagebuch (Hamburg)*, 58–75 (1954)
115. Manning, A., *Behaviour*, **9**, 114–39 (1956)
116. Manning, A., *Behaviour*, **9**, 164–201 (1956)
117. Manning, A., *Proc. Roy. Phys. Soc.*, **25**, 67–71 (1957)
118. Matthes, E., *Z. Tierpsychol.*, **10**, 12–24 (1953)
119. Mayer, G., *Z. Tierpsychol.*, **9**, 337–62 (1952)
120. Mayer, H., *Zool. Jahrb. Abt. Systematik Ökol. u. Geograph.*, **85**, 6, 501–71 (1957)
121. Milum, V. G., *Am. Bee J.*, **95**, 97–104 (1955)
122. Mittelstaedt, H., *Verhandl. deut. zool. Ges. (Freiburg)*, 102–6 (1952)
123. Moore, N. W., *Behaviour*, **4**, 85–100 (1952)
124. Nielsen, E. T., *Spolia Zool. Museum Hauniensis*, **7**, 1–174 (1945)
125. Nielsen, E. T., and Nielsen, A. T., *Ecology*, **34**, 141–56 (1953)
126. Oberholzer, R. J. H., and Huber, F., *Helv. Physiol. et Pharmacol. Acta*, **15**, 185–92 (1957)
127. Pain, J., *Ins. Soc.*, **3**, 199–202 (1956)
128. Pardi, L., *Behaviour*, **1**, 138–72 (1947)
129. Pardi, L., *Colloq. intern. centre natl. recherche sci. (Paris)*, **34**, 183–97 (1952)
130. Perdeck, A. C., *Behaviour*, **12**, 1–75 (1957)
131. Peters, H. M., *Naturwissenschaften*, **27**, 777–86 (1939)
132. Peters, H. M., *Z. Naturforsch.*, [b]**2**, 227–33 (1947)
133. Peters, H. M., *Z. Naturforsch.*, [b]**6**, 90–107 (1951)
134. Peters, H. M., *Experientia*, **9**, 183–88 (1953)

135. Peters, H. M., *Z. Morphol. Ökol. Tiere,* **42,** 278–306 (1953)
136. Peters, H. M., *Z. Naturforsch.,* [b]**8,** 355–70 (1953)
137. Petersen, B., Lundgren, L., and Wilson, L., *Behaviour,* **10,** 324–39 (1956)
138. Petersen, B., Törnblom, O., and Bodin, N. O., *Behaviour* **4,** 67–84 (1952)
139. Piepho, H., *Z. Tierpsychol.,* **7,** 424–34 (1950)
140. Precht, H., *Z. Tierpsychol.,* **9,** 207–30 (1952)
141. Renner, M., *Z. Tierpsychol.,* **9,** 122–54 (1952)
142. Renner, M., *Naturwissenschaften,* **42,** 540–41 (1955)
143. Renner, M., *Naturwissenschaften,* **42,** 589–91 (1955)
144. Ribbands, C. R., *The Behaviour and Social Life of Honeybees* (Bee Research Association, Limited, London, England, 352 pp., 1953)
145. Sakagami, S. F., *Japan, J. Zoöl.,* **11,** 579–91 (1956)
146. Sakagami, S. F., *J. Fac. Sci. Hokkaido Univ.,* [VI]**12,** 333–61 (1956)
147. Sakagami, S. F., *J. Fac. Sci. Hokkaido Univ.,* [VI]**12,** 443–55 (1956)
148. Schmidt, R. S., *Evolution,* **9,** 157–81 (1955)
149. Schmidt, R. S., *Behaviour,* **8,** 344–56 (1955)
150. Schmidt, R. S., *Behaviour,* **12,** 76–94 (1958)
151. Schneirla, T. C., *Colloq. intern. centre natl. recherche sci. (Paris),* **34,** 247-69 (1950)
152. Schneirla, T. C., *Ins. Soc.,* **1,** 29–41 (1953)
153. Schneirla, T. C., *Ins. Soc.,* **3,** 49–69 (1956)
154. Schneirla, T. C., *Smithsonian Repts., No. 455,* 379–406 (1956)
155. Schneirla, T. C., *L'instinct dans le comportement des animaux et de l'homme,* 387–452 (Masson & Cie., Paris, France, 796 pp., 1956)
156. Schneirla, T. C., *Proc. Am. Phil. Soc.,* **101,** 106–33 (1957)
157. Schneirla, T. C., Brown, R. Z., and Brown, F. C., *Ecol. Monographs,* **24,** 269–96 (1954)
158. Schwinck, I., *Z. vergleich. Physiol.,* **35,** 167–74 (1953)
159. Schwinck, I., *Z. vergleich. Physiol.,* **37,** 19–56 (1955)
160. Schwinck, I., *Z. vergleich. Physiol.,* **37,** 439–58 (1955)
161. Schwinck, I., *Z. angew. Entomol.,* **37,** 349–57 (1955)
162. Spieth, H. T., *Behaviour,* **3,** 105–45 (1951)
163. Stride, G. O., *Brit. J. Animal Behaviour,* **4,** 52–68 (1956)
164. Sturm, H., *Z. Tierpsychol.,* **13,** 1–12 (1956)
165. Sudd, J. H., *Brit. J. Animal Behaviour,* **5,** 104–9 (1957)
166. Tinbergen, N., *The Study of Instinct* (The Clarendon Press, Oxford, England, 228 pp., 1951)
167. Tinbergen, N., Meeuse, B. J. D., Boerema, L. K., and Varossieau, W. W., *Z. Tierpsychol.,* **5,** 182–226 (1942)
168. Tsuneki, K., *Mem. Fac. Liberal Arts Fukui Univ.,* [II]**6,** 77–172 (1956)
169. Tsuneki, K., *Mem. Fac. Liberal Arts Fukui Univ.,* [II]**7,** 1–116 (1957)
170. Van Iersel, J. J. A., *Trans. Intern. Cong. Entomol., 9th Meeting (Amsterdam, Netherlands, 1591),* **1,** 384–93 (1952)
171. Van der Kloot, W. G., and Williams, C. M., *Behaviour,* **5,** 141–56 (1953)
172. Van der Kloot, W. G., and Williams, C. M., *Behaviour,* **5,** 157–74 (1953)
173. Van der Kloot, W. G., and Williams, C. M., *Behaviour,* **6,** 233–55 (1954)
174. Vleugel, D. A., *Trans. Intern. Congr. Entomol., 9th Meeting (Amsterdam, Netherlands, 1951),* **1,** 402–8 (1952)
175. Vogel, G., *Z. Tierpsychol.,* **14,** 309–23 (1957)

176. Von Frisch, K., *Naturwissenschaften*, **35**, 12–43 (1948)
177. Von Frisch, K., *Naturwissenschaften*, **38**, 105–12 (1951)
178. Von Frisch, K., *Bayr. Akad. Wiss.*, 197–99 (1953)
179. Von Frisch, K., Heran, H., and Lindauer, M., *Z. vergleich. Physiol.*, **35**, 219–45 (1953)
180. Von Frisch, K., and Jander, R., *Z. vergleich. Physiol.*, **40**, 239–63 (1957)
181. Von Frisch, K., and Lindauer, M., *Naturwissenschaften*, **42**, 377–85 (1955)
182. Von Frisch, K., and Lindauer, M., *Ann. Rev. Entomol.*, **1**, 45–58 (1956)
183. Von Hörmann-Heck, S., *Z. Tierpsychol.*, **14**, 137–83 (1957)
184. Vowles, D. M., *Brit. J. Animal Behaviour*, **3**, 1–13 (1955)
185. Vuillaume, M., *Ins. Soc.*, **4**, 113–56 (1957)
186. Weaver, N., *Ins. Soc.*, **3**, 537–49 (1956)
187. Weaver, N., *Ins. Soc.*, **4**, 43–57 (1957)
188. Weidmann, U., *Rev. suisse zool.*, **54**, 502–11 (1951)
189. Weih, A. S., *Z. Tierpsychol.*, **8**, 1–41 (1951)
190. Weis-Fogh, T., *Phil. Trans. Roy. Soc. (London)*, [B]**239**, 553–83 (1956)
191. Wiedbrauck, J., *Z. Tierpsychol.*, **12**, 176–202 (1955)
192. Witt, P. N., *Behaviour*, **4**, 172–89 (1952)
193. Witt, P. N., *Die Wirkung von Substanzen auf den Netzbau der Spinne als biologischer Test* (Springer Verlag, Berlin, Germany, 77 pp., 1956)
194. Wittekindt, W., *Naturwissenschaften*, **42**, 567–68 (1955)
195. Zippelius, H. M., *Z. Tierpsychol.*, **6**, 372–90 (1949)

EXPERIMENTAL HOST-PARASITE POPULATIONS[1,2]

By T. Burnett
Entomology Laboratory, Belleville, Ontario, Canada

It has been said that man depends on the top six inches of soil for his existence. There is little doubt that his chief competitors for this resource, and the flora which it supports, are the insects. As only a relatively small number of insects are of economic importance, attention has centered in these species; but if we are to understand why one species is numerous and another scarce it is necessary to develop a set of principles which will explain the regulation of the numbers of all species. The growing scientific and practical interest in this fundamental problem of biology is indicated by many recent reviews, such as those by Thompson (59), Solomon (55), Nicholson (42, 43, 44), Milne (40), Huffaker (31), Huffaker & Kennett (33), Varley (72), Cole (19), Andrewartha (3), Birch (8), Utida (70), Andrewartha & Birch (4), Voûte (74), Hinton (30), and Cloudsley-Thompson (18). In addition, the mathematical theory of population regulation has been expanded by Bartlett (6), Wangersky & Cunningham (75, 76), Brian (10), and Williamson (79).

Regulation of insect numbers has been considered, in most theories, in terms of the agents of mortality and how they vary with variations in host density. Although the relative significance of mortality attributable to each of many environmental factors (and consequently the question of whether populations are real entities or statistical concepts) is controversial, it is evident that entomophagous insects are mortality agents of primary importance for they have, over a period of time, the property of increasing the percentage of mortality as host density increases. The relation between host and parasite populations has two apparently contradictory phases: stability and variability. Both host and parasite species tend to maintain average densities over a long period. The numbers of each also fluctuate around the average for a number of reasons: notably discontinuities in life histories, seasonal variations in weather, sudden changes in the physical environment, and host-parasite interaction.

The widespread use of entomophagous insects in attempts to reduce permanently the average densities of some insect species resulted in much theoretical and practical investigations on interactions of herbivores and carnivores and on effects of variation in the physical environment on these

[1] The survey of literature pertaining to this review was completed in February, 1958.

[2] Contribution No. 3818, Entomology Division, Science Service, Department of Agriculture, Ottawa, Canada.

interactions. The consequences of herbivore-carnivore interaction by itself depends, of course, on the particular parameters of the system under study. That variation in the physical environment can greatly modify the efficiency of entomophagous insects in limiting their host numbers is recognized, in one form or another, in nearly all theories on natural control [Thompson (58), Milne (40), Andrewartha & Birch (4), Bodenheimer & Schiffer (9), Smith (51), Nicholson (42), Clausen (15, 16), Michelbacher & Leighly (38), and DeBach, Fisher & Landi (22)]. The manner in which variation in climate may affect natural host-parasite or predator-prey populations may be itemized as follows: climatic extremes (temperature and humidity) often reduce parasite more than host populations [Smith & Armitage (53), Clausen (17), Pickles (49)]; variation in the microclimate modifies the ability of parasites to find and attack their hosts [Voûte (74), Jaynes (34)]; variation in climate from place to place in the same year and from year to year in the same place during the period of population growth affects densities of hosts and parasites [Thompson & Parker (60), Beirne (7), Clausen (15)]; slight differences in climate between the area to which a parasite is transferred and that of its origin often result in asynchronization of their life histories [Helson (29), Clausen (15), Smith (52)]; seasonal fluctuations in temperature and humidity have differential effects on rates of growth of host and parasite populations [Ullyett (61), Andrewartha & Birch (4), Arthur (5), Solomon (54)].

Relation of Experimental to Natural Populations

The study of mortality factors in natural populations usually begins by determining the percentage of destruction attributable to each agent and then ascertaining how this percentage varies with host density. For example, Morris (41) examined variation in host mortality by means of life tables of the spruce budworm, *Choristoneura fumiferana* (Clemens). When the composition of total mortality has been determined, the characteristics of particular agents may be examined in greater detail. The effects of an insect parasite or predator species may be estimated by removing it from the community and recording the abundance of the host in its absence [DeBach (21)]. Or, as is more usually the case, detailed observations may be made on changes in the numbers of parasites and of their host. Most studies listed above were of this type and, of course, many other examples could be given. The results indicate that annual or seasonal variation in climate causes variation in the abundance of host and parasite, and that the influence of the physical environment is probably exercised by its differential effect on survival, behaviour, and development of host and parasite populations. The effect is relatively direct in the case of survival or asynchronization, but where biotic interactions predominate many processes usually occur simultaneously. Studies on the regulation of natural populations, therefore, usually proceed from examination of complex and variable

events to consideration of critical but simpler relationships, and in most cases the interpretations are based on the degree of agreement between observed values and those expected from assumed relationships.

There is no doubt that recording mortality and its causes in natural populations will remain the primary approach to the problem of the regulation of insect numbers. In most comprehensive field studies, however, analysis of mortality eventually becomes concerned with a number of types of interaction between the species studied and environmental factors. For example, Ullyett (63), in an intensive investigation of mortality in populations of *Plutella maculipennis* (Curtis), stated: "It is clear that we require to know much more about the interactions and changes taking place in the environment before we can draw any reasonable conclusions." It is necessary in population ecology, as in any other science, to know the properties and consequences of the elementary processes that occur before more complex events can be understood.

The interaction of host and parasite populations provides a suitable means of beginning the analysis of how the abundance of insects is governed for the following reasons: the experimental material covers the first three trophic levels of the community from which it was removed; the food of the host insects can be varied both qualitatively and quantitatively; the three fundamental aspects of population ecology—natality, mortality, and dispersal—can be examined for both host and parasite and the effects of variation of each may be determined by systematically varying their values; finally, entomophagous insects are considered to be one of the chief density-governing agents by Smith (51) and, as shown above, their properties (as well as those of the host) are greatly modified by variation in temperature and humidity. It should be possible, therefore, to determine the consequences of host-parasite interaction by itself and also to determine which phases of this interaction are critical. Additional variables may be introduced subsequently in a controlled manner so that their influence in modifying this interaction may be observed. This analytical approach, then, is one of proceeding from simple to more complex situations and is chiefly concerned with indicating not only the result of a given relationship but, more importantly, the means by which this result is produced. Thus, experimental populations not only have empirical significance but also provide a basis for the test of certain segments of deduction.

Effect of Physical Environment on Host-Parasite Population

Many studies on laboratory populations have supported the concept that the physical environment affects discrete phases of host-parasite interaction. One example is the effect of climatic extremes on the survival of host and parasite populations. In this connection, Lord & MacPhee (36) investigated the natural control of the oystershell scale, *Lepidosaphes ulmi* (Linnaeus), by the hymenopterous parasite *Aphytis mytilaspidis* (LeBaron)

and the predaceous mite *Hemisarcoptes malus* (Shimer) in Nova Scotia and New Brunswick. The winters in Nova Scotia are usually mild and the host was controlled at an economic level by the parasite and the mite. In the coastal region of New Brunswick temperatures range as low as −16°F. to −20°F. every year or so. This was a marginal area for survival of the parasite and there was some variation in the size of the overwintering parasite population. As the parasite probably had three generations per year, enough parasites survived the winter to re-establish the parasite population and, therefore, both parasite and predator were important agents of host mortality. In central New Brunswick, however, low winter temperatures were −20°F. or lower, and there was no significant increase in parasite numbers in the summers. Hence parasitism was unimportant and the predaceous mite was the main biotic agent of control. This temperature effect was confirmed in the laboratory by exposing the host, parasite, and predator to different low temperatures for different periods of time. Almost 100 per cent of *A. mytilaspidis* were dead within one-half hour after exposure to −20°F. At all experimental temperatures the eggs (the overwintering form) of the host were much more resistant—as measured by the number of hours needed to produce 90 per cent mortality—than were the larvae of the parasite. The mite was very cold-hardy. These laboratory and field studies indicated that minimum temperatures determined which biotic agent regulated the numbers of the scale. A similar type of effect was reported by DeBach, Fisher & Landi (22) in field and laboratory investigations of variation in the success with which *Aphytis lingnanensis* Compere regulated the numbers of its host *Aonidiella aurantii* (Maskell) in different climatic zones of southern California.

The differential rate of development of host and parasite populations at constant temperatures and humidities is without doubt the most extensively investigated aspect of host and parasite relations. Payne (47, 48), in addition to reviewing the early literature, estimated the rate of increase (before the advent of the concept of the intrinsic rate of increase) of two strains of the Mediterranean flour moth, *Anagasta kühniella* (Zeller), and its parasite *Bracon hebetor* Say throughout their temperature range. Ahmad (1) estimated the influence of temperature and humidity on the rate of increase of two strains of *A. kühniella* and another species of parsite, *Idechthis canescens* (Gravenhorst). As a general rule, the relations of host and parasite may be summarized as follows: hosts have a slightly lower threshold of development than parasites, and at low temperatures host populations develop more rapidly than parasite populations, whereas at high temperatures the reverse is the case. An exception to this general rule is given by Ahmad & Ghulamullah (2) for *Earias fabia* (Stoll) and its parasite *Bracon greeni lefroyi* Dudgeon & Gough. In this instance it was estimated that at low temperatures the parasite develops more rapidly than the host and at high temperatures the host population increases faster.

The differential effect of temperature on rate of increase of host and

parasite populations is particularly important in limitation of the numbers of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), by the chalcid parasite *Encarsia formosa* Gahan. In practice, the parasite is unable to control its host during the winter months when greenhouse temperatures, because of the short length of day, are relatively low. With the higher temperatures of the early spring the parasite quickly reduces host density. The first worker to use this agent of control, Speyer (56), noted that the parasite was effective at relatively high temperatures. A program to control growth rates of populations of these species throughout the autumn, winter, and spring months by manipulation of temperature (and of host plants) was formulated by McLeod (37). Later, Milliron (39) exposed host scales on host plants to large parasite populations for periods (apparently) up to one month at different temperatures and recorded the percentages of parasitized hosts. The parasite was most efficient at temperatures of 75°F. to 79°F. When hosts and parasites were reared continuously for several generations, the host outbred the parasite at temperatures slightly below 74°F., and above this temperature the parasite prevented the uninterrupted increase of the host [Burnett (11)]. This relation was governed by differential effects of temperature on such aspects of the rate of increase as fecundity, rate of oviposition, and rate of development of the two species. Where parasites are the principal agent of mortality, as for the greenhouse whitefly, the parasite population must increase more rapidly than that of the host if the host population is to be limited. It is possible, however, for the parasite to have a lower potential rate than the host and yet act as a density-governing factor if parasitism accounts for only a relatively small portion of the total mortality.

The difference in response of a host, and of its entomophagous enemies, to factors of the physical environment is closely related to the factors that regulate their numbers, as the distribution in space of the one usually does not coincide with those of the others. This aspect of host-parasite relations is very difficult to analyze because the differences involved are often very subtle and, in addition, one would also expect that host-parasite interaction itself would lead to differences in the dispersion of the species. An indication of the possibilities in this field is provided by an investigation of the temperature preferendum of the chalcid parasite *Dahlbominus fuscipennis* (Zetterstedt), a parasite of the European spruce sawfly, *Diprion hercyniae* (Hartig) [Wilkes (78)]. The preferendum of laboratory-bred stocks of this parasite was drastically lowered by selective breeding. The preferendum curve for the selected stock was similar to that of parasites collected in the Gaspé peninsula, Quebec, where they had been released some years previously, and presumably the lower preferendum was selected by natural processes. There was great variation in the percentage of hosts parasitized in different areas, and, because of this, Wilkes suggested that laboratory-reared parasites may have preferenda that are unsuitable for some climatic zones.

Effect of Biotic Interactions on Host-Parasite Relations

Studies on the differential toleration and developmental physiology of host and parasite species have been concerned with direct and relatively simple processes. The interactions between host and parasite populations, on the other hand, deal with effects whose results are quite variable and whose causes are complex and sometimes subtle. It is not remarkable, then, that variation in general conclusions on the consequences of host-parasite interaction may arise from the interpretation of particular types of experimentation.

Distribution of parasite progeny among hosts—As it has been difficult to propagate host-parasite populations in the laboratory for an appreciable length of time without providing some form of host protection, attention has centered on some of the postulates used in developing theories of host-parasite growth form. The principal problem in this respect has been the manner in which the parasite population searches for its host. Nicholson & Bailey (45) considered (*a*) that the searching of insect parasite populations is random even if searching by individuals is systematic, and (*b*) that (for simplicity) the measure of efficiency in searching for a given host—called the "area of discovery" or "procurement field"—in a given environment is a constant. These assumptions are strongly criticized by, for example, Andrewartha & Birch (4), who used the data of Ullyett (62, 64, 65) on the distribution of progeny by the parasite *B. hebetor, Cryptus inornatus* Pratt, and *Chelonus texanus* Cresson. Ullyett concluded that the parasites had a strong tendency to avoid hosts that had already been parasitized; that the efficiency of each female decreased markedly as more females searched for a constant number of hosts, chiefly because of a great increase in interference between individuals when searching and ovipositing; and that the percentage of hosts attacked decreased with increasing host density, i.e., the "area of discovery" was not a constant.

Many workers in the field and in the laboratory have demonstrated that insect parasites discriminate to varying degrees between parasitized and unparasitized hosts and also refrain from ovipositing in hosts already attacked. This results in more hosts being parasitized than would result from a random distribution of a given number of parasite eggs among a given number of hosts. Ullyett's criterion of the pattern of progeny distribution, however, is that, of all hosts parasitized, most received a single egg and therefore the female parasites tended to avoid parasitized hosts. But the numbers of hosts receiving 0, 1, 2, 3 . . . eggs can be compared with a random distribution by the formula of Stoy (57), when the total number of hosts exposed to the parasites is estimated in some cases from the tables of Ullyett's papers. In some experiments, the parasite concentrated its progeny in certain hosts rather than avoid parasitized hosts. But whether the parasites underdisperse, oviposit at random, or overdisperse their progeny is not too important. At the densities of host and parasite encountered in experimental populations, the range of parasite progeny per host probably

is limited. Therefore, over the range of superparasitism expected, the distribution of parasite progeny among hosts probably varies from a random one in a limited and systematic manner. In this case, variations in intensity of superparasitism, with variations in densities, probably modify the level of parasitism slightly and in an approximately linear manner.

Up to this point, the relations considered between host and parasite have not been influenced by the area over which the interaction occurred. The intensity of biotic interactions, on the other hand, is determined by (in addition to the physical environment) density relationships of host and parasite. The concern with density, however, has generally led to the neglect of the fact that area of interaction is an important parameter. The main criticism of the experiments of Ullyett is that the hosts were distributed over a very small area (except in one experiment where host and parasite densities were uniformly high) in relation to the powers of dispersal of the parasites, so that intead of searching for hosts, the parasites would have had difficulty in avoiding them. In this case, the important factors of the interaction were discrimination between parasitized and unparasitized hosts, restraint in oviposition, interference among female parasites, and failure of the supply of parasite eggs to keep up with the increase in the number of hosts. If much larger areas had been used, the ability to find hosts would have been important and it is likely that the relation between percentage parasitism and host density would have been greatly modified.

Varley (71) indicated that there were various possible relationships between percentage mortality and population density. One relation was found during a study of effect of host distribution on the efficiency of *E. formosa,* an internal parasite of the greenhouse whitefly. When parasites, 1 mm. long, searched for hosts over flat surfaces, 25 and 100 sq. in. in area, they tended to parasitize hosts in proportion to the number exposed [Burnett (12, 13)], although the percentage parasitism was lower on the larger area. But when the parasites searched over a 200-sq.-in. area, the percentage parasitism was not a constant. With initial increase in host density an increasing percentage of hosts was found, and with further increase this percentage became approximately a constant. This relationship is the opposite to that recorded by Ullyett, since with increasing, low densities the efficiency of *E. formosa* increased rather than decreased.

It is clear, then, that before the postulates of theory are tested adequately it is necessary to use sufficiently large areas of host-parasite interaction. Even so, in practice these relations are not likely to have the simplicity and uniformity that are assumed in mathematical investigations. Thus, the exact growth form predicted for host-parasite populations by the equations of Nicholson & Bailey will not, in all probability, occur in laboratory populations. But empirical investigations have not as yet been conducted on the degree of variation from postulates that is necessary to alter significantly the type of growth form expected from host-parasite interaction. In this connection, Nicholson (42), using numerical examples, stated that

host-parasite "oscillations grow in amplitude with time even when a considerable fraction of surviving animals live in protected situations."

Predator-prey populations.—The classical experiments on oscillations of herbivore-carnivore populations were made by Gause and his co-workers (25, 26, 28) to test the mathematical model of Volterra. Three types of predator-prey populations were used: the protozoan *Didinium nasutum* Müller preying on *Paramecium caudatum* Ehrenberg; *Paramecium bursaria* Ehrenberg feeding on the yeast *Saccharomyces* sp.; the acarine predator *Cheyletus eruditis* (Schrank) attacking the stored-product mite *Acotyledon agilis* (Canestrini). Each of these populations was propagated in small glass tubes that provided a uniform environment. The results may be summarized as follows: except where the eaten species was provided with protection—such as sediment on the bottom on the tube—the prey were quickly eliminated; oscillations were obtained (*a*) when the yeast cells formed a sediment on the bottom of the tube and thus some prey were protected from attack, and (*b*) when there were periodic immigrations of predator and prey. The conclusion follows that some of the prey needed to be protected from attack by the predator, either through shelter or immigration from outside the experimental universe, for the two species to co-exist and their numbers to fluctuate.

A recent study of acarine predator-prey populations is that of Huffaker & Kennett (33) on the economic control of the mite *Tarsonemus pallidus* Banks, a pest of strawberry plants, by the predaceous mite *Typhlodromus* sp. As part of this investigation, two groups of the pest were propagated on two groups of 36 host plants in the greenhouse for 1 yr., in the absence and in the presence of the predator. The growth form of the predator-free populations of *T. pallidus* was one of fluctuations of decreasing amplitude that resulted from a combination of intraspecies competition and a gradual decline in the numbers of mites that the host plants could support. The interaction of predator and prey also resulted in oscillations, but the average density of the prey was much lower than in the predator-free population. There were two successive oscillations, which were regular and small with a reciprocal relation existing between the numbers of predator and prey. The authors considered that two factors modified the variation in numbers of predator and prey. The ability of the predator to feed on honeydew when the prey was scarce allowed *Typhlodromus* sp. to survive, but not to reproduce, at low prey densities; and predation did not reduce prey densities below a certain minimum value because individuals of *T. pallidus* were protected from the predator to varying degrees, by hairs, spines, and leaf crevices of the host plant.

These two examples suggest that oscillations of predator and prey populations follow those predicted by theory but that additional factors, such as protection or partial protection of some prey individuals, are necessary to achieve the result. In Gause's experiments, however, the size of the experimental universe was far too small, even though the interacting organisms

were very small, and it was inevitable that the prey would be eliminated unless they were protected from predation [Nicholson (42)]. In the absence of data, one can speculate that in the *Tarsonemus-Typhlodromus* population the prey would have been eliminated in a small confined area, as there was not (apparently) absolute prey protection. This speculation is supported, to some extent, by the report of Huffaker (32) that, with an unnamed laboratory acarine predator-prey population, the prey was eliminated after an initial fluctuation of prey and predator, and that, when a larger area was simulated by using a more complex universe with barriers to increase further relative dispersion, the growth form was extended to three successive oscillations. It is likely, therefore, that if universes having sufficiently large areas for predator-prey interaction can be devised in the laboratory, the use of immigration and prey protection will not be necessary to provide for the co-existence of predator and prey species.

Host-parasite populations.—As biological control of pests has demonstrated the economic importance of insect parasites, it is not remarkable that the characteristics of these agents of mortality have been examined in some detail. Insect parasites differ from most parasites in that they usually kill their hosts, and from most predators in that only one contact with a host is needed to produce one offspring. In addition, because of the life histories of insect hosts and parasites, the parasite population does not respond immediately to an increase in the density of its host. Nicholson & Bailey (45) deduced that the growth form, initially, of host and parasite populations, in contrast to that of classical predator-prey populations, was one of oscillation around an average density (steady state) with cycles of increasing amplitude. These growth curves and the postulates on which they were based have provided the basis for most of the empirical investigations of host-parasite populations.

Propagation of host and parasite populations usually leads to elimination of one or the other species, depending on the environmental conditions. Flanders (24), however, maintained the Mediterranean flour moth, *A. kühniella,* and its parasite *I. canescens* in equilibrium. The hosts developed in petri dishes filled with food. Balance of host and parasite populations resulted from the fact that the ovipositor of the parasite could be inserted down into the food from the exposed surface for only a short distance. All of the hosts that could be reached by the ovipositor were parasitized, whereas those that were further down in the food escaped and produced moths. Utida and his co-workers, in a long series of papers, analyzed the growth forms of various combinations of two weevils, *Callosobruchus chinensis* (Linnaeus) and *Callosobruchus maculatus* Fabricius, and their parasites, the pteromalid *Neocatolaccus mamezophagus* Ishii & Nagasawa and the braconid *Heterospilus prosopidis* Viereck [see Utida (66 to 69) for references]. Each host-parasite population was reared in a petri dish containing 10 gm. of beans (50 to 60 seeds) and the food was changed periodically. Oscillations of increasing magnitude were not obtained, and Utida con-

cluded that the theory of Nicholson & Bailey did not take into account the effect of density of the host on its own reproductive rate. That intraspecific competition of the host, as well as parasite activity, limited growth of the host population is not remarkable, for the numbers of host and parasite were often in the hundreds. Under these circumstances it is certain that the host had some form of protection from the parasite population, as otherwise it would have been quickly eliminated in such a confined area. The form of this protection is not clear but it might result from the technique, which would allow some hosts to pass through their susceptible stage in the absence of the parasite; or perhaps the parasite females would not oviposit in areas that had been traversed previously. DeBach (20) reported that the latter situation arose with *Mormoniella vitripennis* (Walker), when the parasites so contaminated the experimental environment by running over it in search of their hosts that eventually the number of hosts "stung" dropped to half of normal and the number of hosts parasitized to much less.

The growth form of a host-parasite population was examined in a larger environment when the greenhouse whitefly and its parasite *Encarsia formosa* Gahan were propagated on tomato plants in a greenhouse (Burnett, paper in preparation). Each of seven populations was reared in a section of greenhouse containing 96 host plants of which half were replaced each month. Another population was reared on 64 plants of which a quarter were replaced every half month. The temperature varied between 74°F. and 80°F. throughout the experiments, which began at the end of August and ended the following April. At the beginning of each experiment, adult hosts and parasites were added to the population gradually to minimize fluctuations attributable to the life histories of the insects. The growth form of each host and parasite population was recorded by counting, twice a week, all the adult hosts and parasites on a total of 16 plants.

In general, the growth forms of the host and parasite populations exhibited fluctuations of increasing amplitude. Although there were three "cycles" in the numbers of adult hosts counted during the experiments, two types of host growth form were recorded. In the first type, the second cycle usually had a double peak and the following minimal portion of the cycle was usually higher than the minimum of the first cycle. Thus the average density of the host appeared to be gradually increasing during the course of these experiments. In the second type of host growth form, the second cycle had a single peak, the minimum of the second oscillation was as low as the minimum of the first cycle and the time required to obtain three peaks was longer than in the first type. Here the average density of the host remained low. It is not known at present whether this difference arose from the manner in which the populations were started or whether it resulted from very slight unobserved differences in some environmental factor, such as temperature. The growth form of the parasite was more nearly uniform in all experiments, especially until the end of the second host peak. It generally followed, with a lag, variations in host density. One experimental

population was begun later in the season than its three replicates. As its fluctuations were out of phase with those of the replicates, the fluctuations of host and parasite, although influenced by the season, appear to be of internal origin.

These preliminary experiments indicate that the initial growth form of freely interacting host-parasite populations with overlapping generations can be one of fluctuations of increasing amplitude. The interaction of host and parasite is such a complex and sensitive variable, however, that many more data are needed before the definitive growth form can be established. It is noteworthy that these populations were propagated in an environment that approaches natural conditions.

Models of host-parasite interaction.—DeBach & Smith ((23) developed a singular technique to compensate for the limited but uniform space usually available to those experimental populations used in testing the conclusion of Nicholson & Bailey (45). The parasite *M. vitripennis* searched for puparia of *Musca domestica* Linnaeus distributed among grains of barley in a closed container. Reproduction in each discrete generation was limited in the experiment by assuming that unparasitized hosts doubled in number and the parasite searched for hosts for only 24 hr. This experimental program was carried on for seven generations, during which the numbers of host and parasite varied in a systematic manner, and the authors concluded that, within the limitations of the experiment, the populations followed closely the predictions of Nicholson & Bailey.

These experiments have been evaluated from several viewpoints. This type of model was criticized by Lack (35) because it was artificial and by Andrewartha & Birch (4) because it could lead only to the predicted results. But the model was a means of determining the consequences of a simple interaction (in which the variables were host and parasite density) by eliminating other natural factors. Watanabe (77), using the weevil *C. chinensis* and the parasite *N. mamezophagus* could not reproduce the growth form predicted by Nicholson & Bailey. It is clear from his Table II and Fig. 1, however, that, even with such low factors of increase as 2, 3, and 4, the host population increased, within seven generations at the most, to much greater densities than that of the parasite. As this occurred, no doubt in a limited area such as a petri dish, the host population must have had a measure of protection, by some mechanism, from the parasite population. The results of Watanabe lend some support to the comments given above on the technique used by Utida. Varley & Edwards (73) reexamined DeBach & Smith's data and found two objections to the experiments. First, the female parasites were supplied with honey for four days before each experiment and under these circumstances resorbed most of their eggs: the data show that the mean number of hosts parasitized—a group of eggs is laid on each host—was not over 1.7 (and usually much lower than this figure) in three sets of experiments. Second, because of the low fecundity the area of discovery was approximately constant over the

lower ranges of host and parasite density but fell to very low values (for example 0.006) when host density was high; consequently there would have been a serious deviation from the growth form predicted by Nicholson & Bailey within the next few generations. In discussing these experiments, one is dealing with what *might* have occurred, so the conclusions cannot be adequately tested. The data of Varley & Edwards show that the success of the parasite in finding hosts increased with increasing numbers of hosts. In this connection, if density relations are ignored and only reproductive potential considered, the peak of the host population would occur in the eighth generation and a marked reduction in hosts would be achieved by the tenth generation if each female parasitized an average of 1.4 hosts in each generation after the experimental ones. Whether or not this value would be achieved in practice remains unknown, but it lies within the potential of the parasite and in the last two experimental generations a value of 1.2 was recorded. If density relations are considered, the value of the area of discovery would probably fall only to 0.040-0.030 (from an average of 0.045) within the next few generations. Therefore, the narrow range of densities (around the steady states of host and parasite), within which the experiment of DeBach & Smith supports the growth form predicted by Nicholson & Bailey, may be slightly greater than Varley & Edwards anticipate, and it is even possible that the numbers of hosts and parasites would pass through one cycle. It should be noted, also, that DeBach & Smith determined their experimental program through a process of trial and error, and if the properties of the parasites had been slightly different —which might have been preferable—the experimental technique and program would have been altered. If, however, the effective reproduction of the parasite had been markedly higher, the host would probably have been eliminated in the limited laboratory environment. In any event, DeBach & Smith based their conclusions on the assumptions that the trend of the first seven generations would continue. In the view of Wangersky & Cunningham (76), if the trend of the first seven generations had continued the growth form would have been one of growing oscillation which would settle down into a limit cycle by the tenth generation. Thus, there have been three distinct and, to some extent, opposed interpretations of the same data.

That it is possible, initially, to obtain oscillations of increasing amplitude was demonstrated with the greenhouse whitefly and *Encarsia formosa* Gahan [Burnett (14)]. In line with the technique of DeBach & Smith, the small parasites (1 mm. long) searched for host scales distributed over a flat surface, 441 sq. in. in area, for 18 hr. The parental generation was begun by exposing 22 hosts to 11 parasites, and the experiment was carried on for 21 generations. During these generations the numbers of host and parasite passed through two cycles, the second peak of each species being higher than the first. It is not yet possible to determine whether the departure from the predicted growth form is significant, because this interaction is very sensitive and slight variations have large effects in subsequent

generations. It is known, for example, that the properties, as measured quantitatively, of host and parasite are influenced by slight environmental variation occurring during the propagation of stock cultures.

If it is impossible to propagate host-parasite populations satisfactorily, models of host-parasite interaction appear to be a suitable means of determining the kind of effect, but not the detailed results, to be expected from variation in host and parasite density and of observing how this effect is modified by additional environmental factors.

Conclusions

Although study of predator-prey and host-parasite populations has been one of the classical problems in biology, it has been limited by the necessity of providing, in practice, for the survival of the species attacked. This situation arose because until recently the theoretical bases for investigations usually dealt entirely with changes in density of host and parasite, with the assumption that the size of the environment was unlimited. In practice, however, space has proved to be an important parameter, and within the last few years attention has centered in means of providing sufficient area for experimental populations so that the consequences of host-parasite interaction are not obscured by additional factors. It has been necessary to use small insects or mites with low powers of dispersal. The effective area of interaction has been greatly enlarged by providing either very many surfaces over which the animals disperse or by providing barriers that reduce the rates of dispersal. There are three reasons why the provisions of sufficient space for host-parasite interaction is necessary. First, there is a minimum area, characteristic for the average population of each species of predator or parasite, that is necessary to prevent the elimination of the species attacked. Second, the fluctuations in densities of populations deviate from the average pattern because of random variation [Bartlett (6)], so that a larger area than the minimum is necessary. Third, fluctuations may be greatly accentuated by planned variation in the physical environment [Nicholson (42)]. In addition to providing sufficient space, it is necessary to propagate host-parasite populations for a remarkably long period. In spite of the relatively short life cycles of the insects or mites used, changes in density often occur rather slowly and this may be taken as an indication of the dynamic balance between the two interacting populations.

The immediate use of experimental populations is to determine the growth forms, in a uniform environment, of freely interacting species—with overlapping generations, if applicable. Most research to date has dealt with the type of fluctuation or cycle that occurs, but a problem of equal or greater importance is that of the average density about which the numbers of host and parasite vary. Once a growth form has been established, it should be possible to evaluate by analytical means the relative importance of discrete phases of the interaction. Also, the addition of

physical factors such as variation in temperature and of biotic factors such as host protection and a program of simulated mortality permits an approach to natural conditions. Therefore, after several kinds of experimental populations with different parameters have been described, it should be possible to establish the characteristics and consequences of the action of a single but important agent of mortality and thus to provide complementary information for the interpretation of data on natural populations.

There is little doubt that more complex experimental populations will eventually be developed. Such an approach has been made by Park (46) in an analysis of interspecies competition between the flour beetles *Tribolium confusum* Jacquelin-Duval and *Tribolium castaneum* (Herbst), when both species were either infected with or free of the protozoan parasite *Adelina tribolii* Bhatia. Also, Gause (27) reported on analytical methods of studying more complex communities of microorganisms by submerging glass plates in natural waters and recording the growth forms of the many species that settle on the surfaces. Species taken from the relatively simple communities based on stored products appear to be almost ideal for a prolonged study of increasingly complex herbivore-carnivore populations, and it is no coincidence that many predator-prey and host-parasite populations have come from this source.

The ultimate purpose in using experimental populations is to provide some factual basis for development of discrete phases of theoretical investigations on regulation of insect numbers. The properties of the interacting species and of their environments are likely to be more complex and variable than those postulated in most mathematical theories [Smith (50)], and consequently the predictions of these relatively simple relationships will not be reproduced exactly in empirical studies. This is not a serious handicap, however, for the problem is not to develop experimental populations that will have the properties necessary to produce the expected results. Instead, theory is tested against the interactions of species removed from natural communities, and, if agreement is not reasonable, a search for additional factors or for variation in existing ones becomes necessary. As host-parasite interaction is a very sensitive variable (when measured quantitatively), until many more data are available empirical investigations will probably deal not with details but with the general trend of host and parasite growth forms.

LITERATURE CITED

1. Ahmad, T., *J. Animal Ecol.*, **5**, 67–93 (1936)
2. Ahmad, T., and Ghulamullah, *Indian J. Entomol.*, **3**, 245–84 (1941)
3. Andrewartha, H. G., *Cold Spring Harbor Symposia Quant. Biol.*, **22**, 219–36 (1957)
4. Andrewartha, H. G., and Birch, L. C., *The Distribution and Abundance of Animals* (The University of Chicago Press, Chicago, Ill., 782 pp., 1954)
5. Arthur, D. R., *Bull. of Entomol. Research*, **35**, 257–70 (1944)

6. Bartlett, M. S., *Biometrika*, 44, 27–42 (1957)
7. Beirne, B. P., *Entomologist's Gazette*, **6**, 21–52 (1955)
8. Birch, L. C., *Cold Spring Harbor Symposia Quant. Biol.*, **22**, 203–18 (1957)
9. Bodenheimer, F. S., and Schiffer, M., *Acta Biotheoretica*, **10**, 23–56 (1952)
10. Brian, M. V., *J. Animal Ecol.*, **25**, 339–48 (1956)
11. Burnett, T., *Ecology*, **30**, 113–34 (1949)
12. Burnett, T., *Can. Entomologist*, **90**, 179–90 (1958)
13. Burnett, T., *Can. Entomologist*, **90**, 225–29 (1958)
14. Burnett, T., *Proc. Intern. Congr. Entomol., 10th Meeting*, **2**, 679–86 (Montreal, Que., Canada, 1956)
15. Clausen, C. P., *J. Econ. Entomol.*, **44**, 1–8 (1951)
16. Clausen, C. P., *Calif. Citrograph*, **37**, 182 (1952)
17. Clausen, C. P., *U. S. Dept. Agr. Tech. Bull. No. 1139* (1956)
18. Cloudsley-Thompson, J. L., *The Entomologist*, **90**, 195–203 (1957)
19. Cole, L. C., *Proc. Intern. Congr. Entomol., 10th Meeting*, **2**, 639–47 (Montreal, Que., Canada, 1956)
20. DeBach, P., *Ann. Entomol. Soc. Am.*, **37**, 70–74 (1944)
21. DeBach, P., *J. Econ. Entomol.*, **48**, 584–88 (1955)
22. DeBach, P., Fisher, T. W., and Landi, J., *Ecology*, **36**, 743–53 (1955)
23. DeBach, P., and Smith, H. S., *Ecology*, **22**, 363–69 (1941)
24. Flanders, S. E., *Ecology*, **29**, 123 (1948)
25. Gause, G. F., *The Struggle for Existence* (The Williams & Wilkins Co., Baltimore, Md., 163 pp., 1934)
26. Gause, G. F., *J. Exptl. Biol.*, **12**, 44–48 (1935)
27. Gause, G. F., *Quart. Rev. Biol.*, **11**, 320–36 (1936)
28. Gause, G. F., Smaragdova, N. P., and Witt, A. A., *J. Animal Ecol.*, **5**, 1–18 (1936)
29. Helson, G. A. H., *J. Council Sci. Ind. Research*, **20**, 17–24 (1947)
30 Hinton, H. E., *Sci. Progr.*, **45**, 11–23 (1957)
31. Huffaker, C. B., *Proc. Intern. Congr. Entomol., 10th Meeting*, **2**, 625–36 (Montreal, Que., Canada, 1956)
32. Huffaker, C. B., *Bull. Ecol. Soc. Am.*, **38**, 62–63 (1957)
33. Huffaker, C. B., and Kennett, C. E., *Hilgardia*, **26**, 191–222 (1956)
34. Jaynes, H. A., *J. Econ. Entomol.*, **47**, 355–56 (1954)
35. Lack, D., *The Natural Regulation of Animal Numbers* (Oxford University Press, London, England, 343 pp. 1954)
36. Lord, F. T., and MacPhee, A. W., *Can. Entomologist*, **85**, 282–91 (1953)
37. McLeod, J. H., *Sci. Agr.*, **18**, 529–35 (1938)
38. Michelbacher, A. E., and Leighly, J., *Hilgardia*, **13**, 103–39 (1940)
39. Milliron, H. E., *Mich. State Univ. Agr. Expt. Sta., Tech. Bull. No. 173*, 1–23 (1940)
40. Milne, A., *Can. Entomologist*, **89**, 193–213 (1957)
41. Morris, R. F., *Can. Entomologist*, **89**, 49–69 (1957)
42. Nicholson, A. J., *Australian J. Zool.*, **2**, 9–65 (1954)
43. Nicholson, A. J., *Ann. Rev. Entomol.*, **3**, 107–35 (1958)
44. Nicholson, A. J., *Cold Spring Harbor Symposia Quant. Biol.*, **22**, 153–73 (1957)
45. Nicholson, A. J., and Bailey, V. A., *Proc. Zool. Soc. London*, 551–98 (1935)
46. Park, T., *Ecol. Monographs*, **18**, 265–308 (1948)
47. Payne, N. M., *Biol. Bull.*, **65**, 187–206 (1933)

48. Payne, N. M., *Ecol. Monographs*, **4**, 1–46 (1934)
49. Pickles, A., *Trop. Agr. (Trinidad)*, **10**, 222–23 (1933)
50. Smith, F. E., *Ecology*, **33**, 441–50 (1952)
51. Smith, H. S., *J. Econ. Entomol.*, **28**, 873–98 (1935)
52. Smith, H. S., *Calif. Citrograph*, **31**, 2–8 (1946)
53. Smith, H. S., and Armitage, H. M., *Calif. Univ. Agr. Exp. Sta. Bull. No. 509* (1931)
54. Solomon, M. E., *Ann. Appl. Biol.*, **33**, 82–97 (1946)
55. Solomon, M. E., *Ann. Rev. Entomol.*, **2**, 121–42 (1957)
56. Speyer, E. R., *Bull. Entomol. Research*, **17**, 301–8 (1927)
57. Stoy, R. H., *Bull. Entomol. Research*, **23**, 215–16 (1932)
58. Thompson, W. R., *Parasitology*, **21**, 269–81 (1929)
59. Thompson, W. R., *Ann. Rev. Entomol.*, **1**, 379–402 (1956)
60. Thompson, W. R., and Parker, H. L., *U. S. Dept. Agr. Tech. Bull. No. 59* (1928)
61. Ullyett, G. C., *Union S. Africa Dept. Agr. and Forestry, Sci. Bull. No. 178* (1938)
62. Ullyett, G. C., *J. Entomol. Soc. S. Africa*, **8**, 123–31 (1945)
63. Ullyett, G. C., *Union S. Africa Dept. Agr. and Forestry, Entomol. Mem.*, **2**, 76–202 (1947)
64. Ullyett, G. C., *Can. Entomologist*, **81**, 25–44 (1949)
65. Ullyett, G. C., *Can. Entomologist*, **81**, 285–99 (1949)
66. Utida, S., *Ecology*, **34**, 301–7 (1953)
67. Utida, S., *Mem. Coll. Agr. Kyoto Univ.*, **71**, 1–34 (1955)
68. Utida, S., *Ecology*, **36**, 202–6 (1955)
69. Utida, S., *Oyô-Kontyu*, **11**, 43–48 (1955)
70. Utida, S., *Cold Spring Harbor Symposia Quant. Biol.*, **22**, 139–51 (1957)
71. Varley, G. C., *Trans. Intern. Congr. Entomol., 9th Meeting*, **2**, 210–14 (Amsterdam, Netherlands, 1951)
72. Varley, G. C., *Proc. Intern. Congr. Entomol., 10th Meeting*, **2**, 619–24 (Montreal, Que., Canada, 1956)
73. Varley, G. C., and Edwards, R. L., *J. Animal Ecol.*, **26**, 469–76 (1957)
74. Voûte, A. D., *Proc. Intern. Congr. Entomol., 10th Meeting* (Montreal, Que., Canada, 1956)
75. Wangersky, P. J., and Cunningham, W. J., *Proc. Natl. Acad. Sci. U.S.*, **42**, 699–702 (1956)
76. Wangersky, P. J., and Cunningham, W. J., *Ecology*, **38**, 136–39 (1957)
77. Watanabe, S., *Botyu-Kagaku*, **15**, 73–79 (1950)
78. Wilkes, A., *Proc. Roy. Soc. (London)*, [B]**130**, 400–15 (1942)
79. Williamson, M. H., *Nature*, **180**, 422–25 (1957)

BIOLOGICAL CONTROL OF WEEDS WITH INSECTS[1]

By C. B. Huffaker
Department of Biological Control, University of California, Albany, California

Man's struggle against weeds is endless. Even today there are millions of acres of valuable lands lost to weeds. Some weeds are just hard to kill. Some, although easily killed, grow on lands too low in value or too inaccessible for control by conventional means.

Biological control has solved some of the worst weed problems so far experienced, there being at least five real triumphs. The object of this method is not eradication, but simply the reduction of a weed to a negligible status. The premise of this approach is not restricted to alien weeds; it is based only upon a mutual dependence in the status of a weed and any insect capable of controlling it [Huffaker (49, 50); Nicholson (75, 76); Wilson (120)]. Earlier students of populations thought that phytophagous insects are only rarely limited by scarcity of their plant food [Bodenheimer (7); Imms (56); Muir (72); Smith (99); Thompson (106); Williams (117)]. But much evidence and opinion to the contrary exist, and has increased in recent years. In 1932 Currie & Garthside (20) stated, "It is certainly true that in nature insects exert a profound influence on the density of many plants." (See also 8, 9, 18, 42, 53, 118, 119, 120, 122.) Regarding this, Huffaker (49, p. 126) considers that if insects are not limited by a shortage of food then generally they do not vitally affect the success or survival of their plant hosts, for if they were capable of so affecting their hosts' success, they would reduce their hosts' numbers to such low levels that they would themselves in turn be limited by the scarcity of food. An insect useful for weed control must be capable, either directly or indirectly, of determining the abundance of its plant host (within an environment otherwise favorable to the weed), and thus of determining its own abundance as an interaction.

The writer's philosophy on population dynamics is in essential agreement with that of Nicholson (75, 76) and Solomon (102). Contrary to some misinterpretations [Andrewartha & Birch (1); Cole (16); Milne (70); Thompson (105)], this view does not underestimate the *importance* of the abiotic forces. It *distinguishes* between the roles of the biotic, or density-dependent, actions and those of abiotic nature acting independently of density. Logical argument could be advanced that the physical factors

[1] The survey of the literature was completed in February, 1958. Special thanks are due C. E. Kennett and the late D. H. Groves for their assistance in the preparation of the manuscript and to the various contributors who readily furnished any information requested (see Literature Cited).

are "primary" determinants, whereas density governed reaction is only "secondary," or, on the other hand, that density induced governing action is "primary." Such argument is semantic only and does not clarify concepts. Huffaker (50) stressed the importance in differentiating between *direct* and *indirect* causes. The distinction could be made even clearer by applying the term "proximate" to those more direct actions related to density governance and the term "ultimate" to the physical features of environment which set potentials or favorability, aside from biotic actions serving to modify or alter the requisite levels. It then becomes clear that these forces have supplementary roles and are not in any sense contrary or mutually incompatible as explanations (see also 49, 53, 75).

The whole environment, biotic and abiotic, may be said to "determine" abundance, but only density governed reaction can "regulate" in any real sense of the word—this always in the sense of adjustment to, or in relation to, the *given* conditions of an otherwise relatively stable capacity of environment.

Tillyard (108, 109) and Sweetman (104) referred to the inverse aspect of biological control of weeds as contrasted to biological control of insects. Although they oriented their statements satisfactorily, the author feels that they did not stress the more fundamental similarities. The principles are the same, although in one instance plants are objects to be protected, whereas in the other they are pests to be controlled.

Interests in the two fields may exist at cross purposes. Pettey (84) cites the example wherein the coccinellid *Cryptolaemus montrouziere* Mulsant, previously introduced into South Africa for control of mealybugs, operated against control of *Opuntia* spp. by *Dactylopius tomentosus* Lamarck (= *D. opuntiae* Cockerell), which was introduced there for control of the prickly pears. Dodd (31) reported that the parasite *Opius tryoni* Cameron, previously introduced to Hawaii for control of the fruit fly *Ceratitis capitata* (Wiedemann), was found parasitizing the pamakani gall fly *Procecidochares utilis* Stone, introduced for control of the weed *Eupatorium adenophorum* Sprengel.

As stated by Huffaker (51), employment of biological control of weeds has been hesitantly approached because of: (*a*) fear that the risks involved are too great as compared with the chances of success, and (*b*) conflict in acceptance of a plant as a weed, coupled with the fact that introduced enemies would be free to move into areas where the plant may be of value. The former reason is losing its force before accumulating evidence of successes and greater assurances against undue risk. Furthermore, preponderance of vested interest may well override a decidedly minor consideration.

The object of this paper is to show that the calculated risk may be very good and the chances of success far from remote. There is no basis for considering biological control of weeds with insects only as a "last resort."

PRINCIPLES AND CONCEPTS

Conflicts of Interest

Since the main feature making a plant a weed is simply that it occurs where it is not wanted, contrary interests are inherent in attempts at control. A plant may be a scourge in one place and of much value in another, or, in the same locale, it may be desired by one group and deplored by another. Resolution of the conflict depends on competent opinion drawn from many fields. For such arbitration, committees representing great breadth of competence may be formed. Considerations should include the probability of future values as well as present ones; minority as well as majority rights; interests of other states or nations; direct or indirect effects upon other plants or animals, or the soil itself [Dodd (28); Huffaker (49); Miller (66); Williams (117); Wilson (119, 120, 123)].

The simpler the land economy of an area, the less are the chances of serious conflicts in interest. Perhaps this explains why the United States of America has been late in entering this field, and why, also, the Government of Australia is cautious about starting projects which might be undertaken readily on some island of less complex resources (28, 66, 123).

A few examples of conflicts in interest may be listed:

Lantana camara Linnaeus.—This plant is a serious pest of grazing lands in Queensland and New South Wales, Australia, but to the banana grower it is useful because it immediately occupies land being "rested" from banana cropping (121) and can be easily removed when planting is resumed. It may also be of value in water and soil conservation. Lastly, there is fear that control of *Lantana* may foster the spread of *Eupatorium adenophorum* Sprengel, which is considered a more serious threat to pastures in parts of eastern Australia (121). Nonetheless, Parham *et al.* (79) consider it a fallacy to view *Lantana* as beneficial on plantations in Fiji.

Opuntia spp.—The urgency for controlling prickly pears in Australia in the 1920's was unchallengeable, and the subsequent success of that work has stood as a landmark not likely to be equalled. However, *Opuntia* spp. are not always considered pests. Use of these plants in Hawaii, South Africa, Madagascar, the United States, and Mexico, as human food, fodder, and a source of water for stock on dry ranges has been reported [Fullaway (36); Huffaker (49); Imms (56); Pettey (85)]. Importation of *Cactoblastis cactorum* (Berg) into the United States has long been denied largely on such grounds, although it has been stated that, in West Texas alone, an area of some 60,000,000 acres of range lands are infested with *Opuntia* and consequently suffer greatly lowered values (21). On spineless cactus plantations in some areas of South Africa it has been necessary to control the cochineal previously introduced against the related pest cacti [Pettey (85)].

Echium plantagineum Linnaeus.—In Australia this plant, although generally considered a weed, has some fodder value in certain areas and during droughts [Wilson (123)].

Ulex europaeus Linnaeus.—In New Zealand gorse was early recognized as a serious pest. Yet, in some sections of that country it is used as hedges and, in its early growth, as a fodder crop. Hence, the insects introduced for its control were arbitrarily restricted to those which might curtail seed production and spread without destroying existing plants [Miller (65)].

Centaurea solstitialis Linnaeus.—In California (U.S.A.) yellow star thistle is a complicated example. It is harmful to range lands and in grain and seed crops. But it is reportedly essential to the maintenance of bees in sufficient numbers to pollinate the nut and fruit crops. The claims in this respect may have been exaggerated, and loss to the bee industry could probable be replaced.

The Risks of Introduction and Host Specificity

In the early years of biological control, criticism took the understandable form of fear that introduced insects would become pests (32, 57, 83). The examples of "remarkable" changes in diet of insects customarily cited do not, however, represent basic changes. The insects cited were not originally of demonstrated specificity (49). Yet, the statement of Williams (117) must be a cornerstone of thinking: "The critical phase of biological control work against weeds is the selection of species that will not harm other plants, or at least useful plants. All other considerations are subordinate. . . ."

Thus, while only insects of very restricted diet can be introduced, it does not follow that no such insects exist nor that it is unsafe to introduce phytophagous insects when full precautions are taken. The question is just how firm the assurance can be. There are two reasons for confidence, one based on academic considerations and the other on a record of results.

Evidence on the nature of specificity shows how very unlikely it is that an introduced insect will become a pest if all reasonable precautions are taken. In 1928, Tillyard (109) stated that the real risk stems from our ignorance on host selection and specificity rather than from the method itself. The risks are related to the degree of specificity of the insects considered and the closeness of the weed, botanically, to plants of economic importance. The conclusion of recent research is not the promiscuous or changeable nature of phytophagy, but rather the "near immutability" and rigidity of such feeding habits [Brues (8, pp. 94-96); see also Dethier (26)]. This subject is summarized with respect to biological control of weeds by Huffaker (49). Although evolution from polyphagy to a restricted diet seems to have been more common, Dethier (27) has proposed that the course may also proceed from monophagy to secondary polyphagy. He also stressed that not only may the insects change their feeding habits by virtue of some form of learning or mutation or both, altering either their behavior or basic physiology, but also that the plants may undergo alteration, gaining qualities which make them acceptable, whereas they previously were not.

Thus, either the potential plant hosts or the insect may change in a

way causing a new food plant to be accepted, but these changes are, as in all evolution, rare, and they are the product usually of great periods of time. Constancy rather than change is the great rule of nature [Huxley (55)]. Dethier (*27*) suggests the possible transition of members of the host-restricted genus *Papilio* from *Citrus* to *Ruta,* which possess different oils, by gradual steps through *Zanthoxylum,* which possesses the phagostimulants characteristic of both *Citrus* and *Ruta.*

Since specialization (in diet or otherwise) is a deepening rut in evolution (55), one may conclude that, while there can be no guarantee that an insect will not accept a new plant, those forms which have long progressed in the rut of high specificity are extremely unlikely to escape from it. Since the insects chosen for biological control are of this type only, the safety of the method is assured.

The folly of assuming that, since there is the ***possibility*** of an insect's adopting new hosts, phytophagous insects should not be introduced was well analyzed by Miller (66) and Wilson (119). Miller stated,

> Nevertheless, if we are to condemn the utilization of specialized phytophagous insects as a factor in weed control on account of this comparatively rare element of danger, and after all human precautions have been taken, then we must be prepared to have our crops overrun by any, or all, of the many species of phytophagous insects as yet harmless.

Wilson pointed out that the chance that a dietary change will cause an introduced species to adopt new hosts is no greater than the chance that such change will occur among our thousands of presently innocuous species.

The second reason for confidence is based on results. Although in the earlier work the conducting of "starvation tests" had not then come into vogue, no insect introduced for control of a weed has become a pest [Dodd (28)]. This assertion excepts very minor feeding on some crop plant [e.g., *Thecla echion* Linnaeus on eggplant in Hawaii (83)] and the fully expected attack on the spineless cactus in South Africa by insects introduced there for control of other forms of these plants growing as pests. (With regard to *T. echion,* the insect was not subjected to starvation tests prior to release in Hawaii. Properly executed tests would certainly have detected such capacity.)

Interpreting "Starvation Tests"

There has been controversy regarding the interpretation of "starvation tests" and the weight to be given such data. Decisions cannot be entirely objective. The urgency of each problem will alter the acceptability of an estimate of risk.

It is unreasonable to insist that an insect be unable to engage in attempted or abortive feeding upon some economic plant even under forced or unnatural stress. The capacity to breed upon a given plant is the proper criterion. In nature there are a great many factors which may prohibit an

insect from using a plant capable of serving it nutritionally. Great weight should be given to the host records of the insect in its native home and to whether or not it has been known to depart from the normally restricted host range. This subject is considered by Huffaker (49, pp. 141-43). Dodd (29, p. 3) stated: "In two or three instances, prickly-pear insects were able to feed and to develop on certain other plants or fruits, although all evidence pointed to their absolute restriction to cacti under field conditions in America." The same author (28) concluded that the whole question is broader than the mere ability or inability of the insect to feed on some plant of economic importance. Williams (117) stated,

> The leaf-eating beetle, *Schematiza cordiae* Barber, was tested in the orthodox manner by starvation tests in both Trinidad and Mauritius before its release in the latter country against *Cordia macrostachya* (Jaquin) Roemer & Schultes. When it was confined in petri dishes, slight feeding upon several unrelated plants occurred, while appreciable feeding occurred upon the weed and its close allies, and also, surprisingly, upon cabbage.

Under field conditions, this insect was entirely unable to breed on other species of *Cordia* or other plants. Dodd (28) listed the following points as relevant to the interpretation of tests:

(a) the importance of the weed, and the difficulty of its control by any method other than the biological;

(b) the potential value of the insect for the control of the weed;

(c) the value of the economic plant which may be attacked by the insect, weighed against the damage and loss of production caused by the weed;

(d) the seriousness or otherwise of the damage that might be caused to the economic plant by the insect's attack;

(e) the simplicity or otherwise of the insect's control by cultural, chemical or other means, if and when it attacked some particular economic plant.

Some Unfounded Criticisms of the Method

During recent years the undoubted success of the method, when applicable, and the repeated demonstration that the work can be conducted with safety, have evoked some criticisms which are largely negative. Such attitudes are often voiced verbally but seldom are seen in scientific literature. They have, nevertheless, great impact upon decisions regarding initiation of such work.

The first such view is that since the method requires no effort on the part of the agriculturist, it promotes complacency and neglect of proper methods of management. Wilson (123) answered: "But this objection cannot apply to weeds for which chemical or mechanical control is used or to those problems for which pasture improvement is not a solution, or to those terrains on which it is impracticable." Both Wilson (121) and Huffaker & Kennett (54) stressed that control by biological means of a pest which previously precluded or dominated research or proper land utilization only reopens the avenues to further improvement.

A second negative view—that if we control a weed biologically we may get a worse one in its place (admittedly possible)—was countered by Huffaker (48):

> With regard to the possible invasion by new weeds of space vacated by Klamath weed [St. Johnswort], we can make no assurances. There are no data indicating that such a species as medusa-head (*Elymus caput-medusae* Linnaeus) shows a marked predilection for space recently vacated by Klamath weed, nor that it is incapable of invading Klamath weed infested land, or land which never at any time has been so infested. There is no reason to assume that there would be any basic difference as to the final plant composition resulting from a control of Klamath weed accomplished by beetles and that accomplished by chemical means. No one can say that some other perhaps more serious weed may not some day lay claim to the land, whether or not Klamath weed is brought under control.

The same author (49, p. 147), while admitting Miller's (66) statement of the possibility that the "other" weed might prove more difficult to control, stated:

> But no one should take a defeatist's attitude and assume per se that an aggressive, alternate weed is worse and certain to claim the space so vacated. There are many reasons why it is unlikely to do so, or, at least, to the same extensive degree, or why it *may* prove easier to control by conventional means, rather than more difficult.

The Probability of Success

The proportion of successes in the biological control of weeds is highly encouraging. Although no problem has yet been fully explored, there have been few, if any, failures where a serious attempt has been made, and at least partial success has attended every real effort [Dodd (28)]. In contrast to Cameron (11), the late H. S. Smith, eminent exponent of biological control, often commented that use of the method in control of weeds offers proportionately greater chances of success than in the control of insect pests, and that a much wider application is justified. The favorable position of biological control of weeds has been summarized by Huffaker (49, p. 133); here the points are presented with some abridgement, and an additional point is contributed by Flanders (33): (*a*) The method has been used only against weeds of undisturbed lands. (*b*) Weeds are engaged in competition with other plants, with the latter being favored by the insect action against the weeds. (*c*) The insect injury may be attended and extended by action of plant pathogens (118). (*d*) With weeds, in contrast to insect pests, there seems to be no general deterrent to effective control by introduced agents in temperate regions contrasted to tropical. (*e*) The work has necessarily been restricted to promising prospects. (*f*) The insects used against weeds are necessarily highly specific, whereas many of those introduced against insect pests are more generalized in diet and hence less apt to be highly effective (33). (*g*) There is also a new factor not at all similar to the situation with insect pests. Plants do not always die from the attack of a single insect, and the greater numbers generated at low host densities would make for greater searching effectiveness.

The fact that our worst weeds are aliens lends encouragement, for their aggressiveness in the new country is often (but not necessarily) caused by the absence of phytophagous enemies. Furthermore, although there are various causes for phenomenal aggressiveness of an alien weed, it is well established that the chance of success in introducing natural enemies increases with the abundance of the pest [Cameron (11); Huffaker (49, pp. 133-37, 139); and others].

Some of the most competent men in the field have at times prejudged certain insects as unlikely control agents, for such reasons as that their damage to the weed in the native region is "apparently" insignificant or that they have a single generation per year or a low fecundity per female. Huffaker considers the particular environment in all its aspects as dominant and feels that insects which exhibit little direct damage to the plant or those which reproduce slowly may, if the right relations exist in the new environment, effectively control a given weed. Presumptive "estimates" of an insects potential can be very wrong. Williams (117) would seem to concur: ". . . insects that do little harm to a plant in its native country may be eminently successful control agents. . . ." The exclusion of an insect because it has a single generation each year has no basis; witness the phenomenal success of *Chrysolina quadrigemina* (Suffrain) (=*Chrysolina gemellata* Rossi and *Chrysomela quadrigemina* Suffrain of some authors) in the control of St. Johnswort in California. It is probable that the arbitrary restriction of this method to weeds of particular habit of growth, type of land infested, or origin (alien or native) has been overemphasized (49, 51).

Textbooks on plant ecology offer no hint of the soundness of biological control of weeds, in that they ignore the role phytophagous insects may have on the composition of vegetation. This omission results from their emphasis on the plant environment and the climatic and edaphic factors, with a consequent neglect of biotic forces of many and varied sorts which may drastically alter utilization of such resources as are otherwise available to given plants. Huffaker (49), Huffaker & Holloway (52), and Wilson (118, 119, 120) have discussed this neglect and documented the effect which insects sometimes exert on either native or alien components of vegetation. Significant action may be obvious or subtle. A review is given here of some insects which were considered to act significantly on certain plants in their natural environments. Fuller discussion of some of these instances will appear under the heading "Examples."

In 1902, Koebele (83) considered that the insects affecting *Lantana camara* Linnaeus in Mexico were very important in determining its vigor, distribution, and rapidity of spread. He also thought that, if not themselves attacked by entomophagous parasites, they would exert a much more decisive influence. Other direct or inferential evidence of general or partially significant effects of insects on the success of plant species in vegetation include:

(*a*) The observations of important effects of insects on St. Johnswort, *Hypericum perforatum* Linnaeus, by A. H. Hamm in 1919, by Garthside in 1926 in England, and by Wilson from 1935 to 1940 in France (20, 118).

(*b*) The influence of insects on the pattern of regeneration of trees in a climax forest in South Africa, reported by Bews (6).

(*c*) Osborn's failure (77) to find significant action of insects on *Eupatorium adenophorum* Sprengel in Mexico, but his suggestive comment, ". . . since I have not seen the plant growing when free from attack, I may possibly underestimate the effect of a retarded growth in competition with other plants."

(*d*) Phillips' (88, 89) conclusions that insects attacking *Olivia, Apodytes, Olea,* and *Ocotea* in Knysna Forest (South Africa) did not alter the climax but seemed ". . . wholly necessary for the keeping of a proper balance in the organic scale, perhaps being responsible for originating *subseral succession.*"

(*e*) Chater's (13) studies of insects attacking gorse in England, and his opinion that the moth *Anarsia spartiella* Schrank probably would be much more significant were it not for the heavy attack it commonly suffers from entomophagous insects.

(*f*) the role of seed- and flower-destroying insects in the natural control of not only *Clidemia hirta* D. Don but also other species of the genus in Trinidad [Simmonds (97)].

(*g*) Janvier's (58) conclusion that native *Acaena* in Chile are only very minor pests because of the severe control of these weeds by insects—one being a sawfly, *Antholcus varinervis* (Spinola).

(*h*) Sweetman's statement (104) that the Australian species, *Acaena sanguisorbae* Vahl, is controlled in some districts by the beetle *Haltica pagana* Blackburn.

(*i*) The thorough studies of Cameron (11) showing the important influence of insects, among other factors, on the ragwort *Senecio jacobaea* Linnaeus in parts of England, particularly the moth *Tyria jacobaeae* Linnaeus.

(*j*) Studies reporting the influence of various insects on the abundance of cacti in the United States, including control by the phycitid *Melitara dentata* (Grote), under favorable conditions on the Great Central Plains (Kansas, Nebraska, etc., U.S.A.) (10, 17, 82) and to some extent in Texas also (21); and reporting that such insects as *Dactylopius confusus* Cockerell (= *D. newsteadi* Cockerell), *Chelinidea vittiger* Uhler, and *Moneilema* spp. would be of greater importance were they not attacked by entomophagous enemies.

(*k*) The assignment by Maehler (63) of a significant role to the tortricid *Spilonota holotrephas* Meyrick, in reducing guava on Guam [Note: Steiner (103) questioned this interpretation].

(*l*) Simmonds' (96) striking experimental proof that the action of entomophagous parasites (*Tetrastichus* sp. and *Chaetonodexodes marshalli* Aldrich) prevented the galerucid *Schematiza cordiae* Barber, from completely defoliating bushes of *Cordia macrostachya* (Jaquin) Roemer & Schultes, in Trinidad.

(*m*) The wholesale transfer and establishment of the mealybug *Eriococcus orariensis* Hoy, on the scrub manuka, *Leptospermum scoparium* Forster, in New Zealand, with good success in control of the pest (not unequivocally a pest, however [Hoy (47), Miller (67), Sewell (95)].

(*n*) Observations on the controlling action of *Gastrophysa cyanea* Melsheimer on dock, *Rumex* spp., in California, at times severely curtailed by the entomophagous wasp, *Microctonus gastrophysae* (Ashmead) (49).

(*o*) Statements of Tisdale & Zappetini (111) that a scale insect, *Orthezia*

annae Cockerell, and a snout moth, *Eumysia* sp., were a partial cause of extensive death of shadschale, *Atriplex confertifolia* Watson, in western United States.

(*p*) Studies in India showing that the cerambycid *Nupserha antennata* Gahan severely damages noogoora bur, *Xanthium* sp., in many areas in that country ". . . giving [it] the appearance of having been reaped" (28).

(*q*) Reports of Pringle (90) and Arnott (3) that sagebrush, *Artemisia tridentata* Nuttall, is sometimes controlled by a beetle, *Trirhabda pilosa* Blake, in the western United States and Canada, with consequent increase in the grass cover.

(*r*) Louis-Marie's (62) report of control of toadflax, *Linaria vulgaris* Miller, in Canada by a beetle, *Gymnaetron antirrhini* Paykull.

(*s*) Thompson's (107) report of the action of the accidentally introduced scale insects *Carulaspis visci* (Schrank) and *Lepidosaphes newsteadi* (Sulc) in nearly annihilating the native Bermuda "cedars," *Juniperus bermudiana* Linnaeus, on some islands of Bermuda, exemplifying the force which an alien insect may exert on native vegetation.

(*t*) Heinze's (41) summary of aphid damage to plants, including many weeds, which restricts their reproductive and general competitive position in nature.

(*u*) The suggestion of both Sweetman (104, p. 382) and Heinze (41)—based upon the common knowledge of phytopathology resulting from such relations—that plant pathogens and the insect vectors associated be introduced to control weeds, provided assurances can be had of the specificity of both the insects and the diseases.

(*v*) The serious depredations of forest trees and other plants by accidentally introduced alien insects that are matters of sad record, although the effects have not commonly been studied in relation to the composition of vegetation subsequently.

It is recognized that opinions and evidence such as those just listed are perhaps biased and certainly incomplete. Long-term, comprehensive studies employing insecticidal check methods for evaluating the role of the highly selective phytophagous insects in the determination of plant cover are sadly needed. The results could be startling to the ecologist.

EXAMPLES OF BIOLOGICAL CONTROL OF WEEDS

Lantana, Lantana camara Linnaeus

Hawaii.—The first attempt at biological weed control[2] was made in Hawaii in 1902 (35, 83), where *L. camara* threatened ranching interests. Perkins (83) stated:

> When one looked over miles of country covered with almost continuous growth of the plant, every bush in its season with masses of flowers and fruit, one might well doubt whether anything could be done to check a growth that had already acquired so strong a hold on the land.

Destruction of this weed by the scale insect *Orthezia insignis* Douglas,

[2] There is actually an earlier example: ". . . the ability of insects to destroy prickly pear had been known and applied in India and Ceylon since cochineal insects had been introduced . . ." in 1795 to develop a dye industry, wrote Wilson (124).

which had arrived accidentally, had been noticed, and ranchers had engaged in establishing it on their ranches. Their efforts gave impetus to the plan of sending Koebele to collect *Lantana* insects in Mexico and Central America for introduction in Hawaii.

Of the insects introduced, eight became established (83): a seed fly, *Agromyza lantanae* Froggatt (=*Ophiomyia lantanae*); a lace bug, *Teleonemia scrupulosa* (Stål) (=*T. lantanae* Distant); a tortricid, *Epinotia lantana* (Busck) (=*Crocidosema lantanae* Busck); a moth, *Platyptilia pusillidactyla* (Walker); a leaf miner, *Cremastobombycia lantanella* Busck; a gall fly, *Eutreta xanthochaeta* Aldrich; and two butterflies, *Thecla echion* Linnaeus and *Thecla bazochi* Godart (=*Thecla agra* Hewitson).

The total area covered by *Lantana* is now much reduced, and anxiety over its spread has been allayed. Perkins & Swezey (83) stated that, since it is not hard to clear this weed off the land, the most valuable insects have been those which prevent seed production and thus curtail its capacity to re-establish itself. He did state that on certain parts of the islands pamakani, *Eupatorium adenophorum* Sprengel, "klu," *Acacia farnesiana* (Linnaeus) Willdenow, and guava, *Psidium guajava* Linnaeus, have come in and have become worse pests than was *L. camara*. However, Davis (24) stated that *Lantana* is still the problem of greatest concern to the rancher, generally.

Fifty years after Koebele's work, introductions were renewed. Krauss (59) reported the importation of a tingid, *Teleonemia vanduzeii* Drake, from Cuba in 1952, and Gardner (39) reported that it was also shipped to Hawaii from Florida by Krauss in 1955-56. It has been released, but the results are yet unknown.

According to Dodd (30) and Davis (25), the following insects were recently released in Hawaii: a cerambycid, *Aerenicopsis championi* Bates, the noctuids, *Catabena esula* (Druce) and *Diastema tigris* Guenée; the pyraustids, *Syngamia haemorrhoidalis* Guenée and *Blepharomastix acutangulalis* (Snellen); and an agrodid, *Hypena jussalis* Guenée, the last species coming from Africa (25). Favorable recoveries of the cerambycid have been made on Hawaii. *C. esula* is now established on Hawaii, Maui and Oahu. *B. acutangulalis* and *D. tigris* have not been recovered. *S. haemorrhoidalis* is well established. Davis (24) considers it very promising—a likely control factor in destroying the new growth following defoliation resulting from attack by *T. scrupulosa*.

Wilson (123) recorded the release in Hawaii of two chrysomelids, *Octotoma scabripennis* Guérin from Mexico and *Octotoma plicatula* Fabricius from Honduras. Davis (24) asserts that a misidentification resulted in the destruction of the original material of the cerambycid *Plagiohammus spinipennis* Thomson but that it is to be again obtained from Mexico and retested for specificity.

Fiji.—Lantana camara was one of the first weeds to become a serious problem in Fiji, where it forms dense thickets, especially on coconut estates and on ranges [Parham *et al.* (79)]. Four insects were introduced via Ha-

waii for its control, but only three, *A. lantanae, T. bazochi,* and *T. scrupulosa* became established. Simmonds (98) reported a consequent material reduction of the weeds. Fiji has also joined the recent effort of Hawaii and Australia, and *B. acutangulalis* and *D. tigris* have been released in Fiji (79).

India.—Gardner (38) noted that *T. scrupulosa* was tested for specificity and found to attack teak, a valuable tree in India of the same plant family as *Lantana*; hence, the work was stopped. Fullaway (35) reported also the introduction via Hawaii of *A. lantanae.*

Australia.—*Lantana* is a pest in tropical and subtropical coastal areas in Queensland and New South Wales (12), where it invades burned-over areas, grazing lands, and forest plantations. Several insects were introduced via Hawaii and Fiji in 1914 and 1917, and *A. lantanae* and *E. lantana* became established but with little result. *T. scrupulosa* was introduced via Fiji in 1935. It was reported in 1946 (12) to cause all stages of host damage to complete defoliation and death of the plants. According to Dodd (30), it is now present throughout Queensland. Attempts to establish it in coastal New South Wales have failed. Cashmore & Campbell (12) stated that it ". . . may be expected to exercise useful control of *Lantana* only in the more tropical regions of Australia." Wilson (124) states that none of these insects appear to have a significant controlling effect, although he relayed a report of the bug's causing widespread defoliation on Norfolk Island.

Although Wilson (123) stated in 1954 that general agreement as to the need for biological control of *L. camara* in Australia did not exist, Dodd reported in 1957 (30) that four Lepidoptera, *B. acutangulalis, S. haemorrhoidalis, C. esula* and *D. tigris* were cleared for release, but none was yet established. The work is continuing.

Prickly Pears, Opuntia spp.

Australia.—This classic example of biological control is so well known that no effort will be made here to give it the coverage its importance merits. The problem in Australia and the ultimate solution were well documented by Dodd (29). *Dactylopius indicus* Green (=*D. ceylonicus n.n.*) was introduced in 1903. Had it become established at that time, the example would have equalled in chronology and perhaps excelled in result the Hawaiian work on *L. camara,* for when this insect did become established in 1913 and 1914, it gave good control of the minor pest, *Opuntia vulgaris* Miller (=*O. monacantha* Haworth). But the major pest species, *Opuntia stricta* Haworth (=*O. inermis* De Candolle), supplemented somewhat by other species in certain districts, continued to spread so widely that by 1925 it affected 60,000,000 acres (and was still increasing rapidly) of which about 30,000,000 had become useless. Because the cost of chemical or mechanical control was greater than the value of the land, a desperate situation was faced.

Beginning in 1920, investigations in the United States, Mexico, and Argentina resulted in the introduction and establishment in Australia of 12 species which attack *Opuntia.* Actually, some 50 species were sent, but Dodd

stated that a number of factors, including the striking success of *Cactoblastis cactorum* (Berg), which rendered further efforts unnecessary, accounted for the fact that only 12 were established. Insects introduced prior to *C. cactorum,* including *Chelinidea tabulata* (Burmeister), *Dactylopius tomentosus* Lamarck, *Olycella junctolineela* (Hulst), and a mite, *Tetranychus desertorum* Banks (=*T. opuntiae* Banks), made rapid progress from 1925 to 1927. From 1930 on, *C. cactorum* mastered the prickly pears and eclipsed all other species. Dodd characterized the period from 1935 to 1940: ". . . the virtual complete control of the major pest pears by *Cactoblastis.* The former dense pear country reclaimed and brought into production. Destruction of tiger pear, *Opuntia aurantiaca* Lindley, by the Argentine cochineal [*Dactylopius* sp., near *confusus*" (*29,* p. 99)]. Bacterial rots and diseases had a secondary role. Wilson (124) reported that substantial or partial control of four of the five minor pest species of *Opuntia* has also been achieved, but that some species are still pests of importance in parts of New South Wales. He added, however, that had prickly-pear insects never been introduced, it would be difficult to visualize the magnitude of the problem which would now face Australia.

The other species of the 12 established against *Opuntias* are: a phycitid, *Tucumania tapiocola* Dyar; three cerambycids, *Moneilema ulkei* Horn, *Moneilema variolare* Thomson, and *Lagocheirus funestus* Thomson; a coreid, *Chelinidea vittiger* Uhler; and a cochineal, *Dactylopius confusus* Cockerell (=*D. newsteadi* Cockerell).

India and Ceylon.—According to David & Muthukrishnan (22), *Opuntia vulgaris* Miller and *O. elatior* Miller (=*O. nigricans* Haworth) were introduced in India about 1787 to culture the commercial cochineal, valued for its dye, while *Opuntia dillenii* (Ker-Gawler) was probably introduced much earlier. The prickly pears became serious weeds over vast stretches of the country and remained so for more than a century and a half. *O. vulgaris* had long been under effective control by the cochineal, *Dactylopius indicus* Green, which apparently arrived along with the introduced *Opuntias.*

The infestations were reduced by the introduction of the cochineal *D. tomentosus,* in 1924-26. Within a couple of years, this scale effected striking control of *O. dillenii* and, to a lesser degree, of *O. elatior.* According to Narayanan (73), about 100,000 acres infested by the former species in India were cleared of the pest.

Celebes.—The prickly pear *O. elatior* was rapidly covering abandoned rice fields and grazing lands in the Palu Valley of northern Celebes in the early 1930's. Because of its previously reported success against this species of *Opuntia, D. tomentosus* was introduced via Australia and liberated in 1935. Van der Goot (112) stated that results were first observed in May 1936, while by late 1939 the dense cactus had been killed everywhere, and many fields were again being tilled or used for grazing.

South Africa.—About 20 species of *Opuntia* occur in South Africa, mostly introduced about the middle of the eighteenth century. The two main

species, *Opuntia megacantha* Salm-Dyck and *Opuntia aurantiaca* Lindley, developed extensive infestations on the veld, rendering much of the land useless. About 1,000,000 acres were infested by *O. megacantha* alone.

O. aurantiaca was introduced around 1850 to 1860. Pettey (84) called it ". . . the worst weed in South Africa." By 1933, he said, ". . . it was looked upon as a national menace." From 1934 to 1938 the government spent over £172,000 in an eradication campaign. Surveys revealed infestations of about 420,000 acres in eastern Cape Province, plus other infestations in the Orange Free State and Natal. But chemical and mechanical methods of control proved too expensive, and in 1932 it was finally decided to attempt biological control by use of *C. cactorum,* which was introduced that year (84).

Five additional insects were studied as a possible supplement to *C. cactorum.* They proved only partially successful because of the resistant nature of the *Opuntia* concerned and of certain biotic and abiotic factors in the environment. However, *C. cactorum* prevents spread of the plant by destroying new plants. The other insects imported were *Cactoblastis doddi* Heinrich in 1934 (not liberated), *D. tomentosus* in 1937, *Lagocheirus funestus* Thomson in 1938, *Moneilema ulkei* Horn in 1939 (not liberated), and *Cactophagus spinolae* (Gyllenhal) in 1952. Only *C. cactorum* and *C. spinolae* became established, the latter showing little promise [Naude (74); Pettey (86); Sellers (94)].

Except for the attacks of an indigenous coccinellid, *Exochomus flavipes* Thunberg, and the previously imported *Cryptolaemus montrouzieri* Mulsant, the cochineal would probably have given complete control of both pest pears. Even under this stress it kills off a large proportion of a *megacantha* plant, and, if it is supplemented with mechanical operations at the critical time, regrowth is prevented and the land reclaimed. In 1950 Pettey (85) stated that by this method 90 per cent of the area originally infested by this species in the Karoo and other parts of the eastern Cape Province had been cleared and that most of the remaining infested area was in a belt within 50 miles of the coast. As a result, sprays of DDT are required to control the coccid in spineless cactus plantations, except in the coastal areas, where the natural enemies check *D. tomentosus* (87). In 1956 Naude (74) published an appraisal of this work. He feels that the program, including supplemental felling operations, resulted in ". . . control over the major part of the infested area, and consequently [the *Opuntia*] no longer presents a problem at the moment." He added that complete control was not achieved in certain patches—particularly in some high-lying and mountainous areas where neither *C. cactorum* nor the cochineal reach their potentials. He stated that in the coastal belt there is a continuing problem. It is his opinion that had supplemental felling been done at the proper moment, now a tragically lost opportunity, control would have been good even in many of the trouble spots.

Hawaii.—The tree cactus, *O. megacantha,* apparently introduced from

Mexico about 1809, has spread alarmingly since about 1900 on the island of Hawaii, covering the lower grazing areas almost solidly and encroaching into the upper and better pastures (36). Objections to introducing insect enemies onto some other islands of the group have not yet been overcome, because of the use that is made of the red-fruited variety by some cattlemen.

According to Fullaway (36), the following insects were received in Hawaii, beginning in 1949: the California cochineal, *D. confusus* (not established); *C. tabulata* (not released); *C. vittiger* (not released); the Mexican cochineal, *D. tomentosus* (established); the moths, *Melitara prodenialis* Walker and *Melitara doddalis* Dyar (neither established), and *C. cactorum* (established); the borers, *Moneilema armata* Le Conte (never recovered); *Moneilema crassa* Le Conte (established ?); and *L. funestus* (probably established). Fullaway (37) and Bess (4) have since reported excellent success with *C. cactorum* and *D. tomentosus*.

Mauritius (2).—In 1927 *D. tomentosus* was introduced via Ceylon into Mauritius for control of the pest pear, *Opuntia tuna* Miller. Subsequently the weed was not very abundant because of control by the cochineal. However, it was reported that in the years just prior to 1950 there seemed to be some increase caused by the predation on the cochineal by *Cryptolaemus montrouzieri* which was imported in 1938 and 1939 for control of the pineapple mealybug. *C. cactorum* was imported via South Africa in 1950 to assure continued suppression of the cactus. The moth is now widespread and the *Opuntia* is frequently seen collapsing from its attack.

St. Johnswort, Hypericum perforatum Linnaeus

This weed, also known as "Klamath weed" in western United States, is a scourge in many temperate regions of the world having moderate rainfall at least during the cold season. It is associated particularly with sheep ranching, but may cause even greater losses on cattle ranges. It is toxic if eaten heavily and may cause losses in weight or death of stock. Yet, by far the greatest loss results from its displacement of desirable forage. It attains a dense cover under favorable conditions, and a sparse, stunted growth under partial shade or other unfavorable conditions.

Australia.—St. Johnswort caused concern in 1917 in Victoria, New South Wales, and elsewhere. In the 1930's from 250,000 to 400,000 acres were infested in Victoria alone. Upon requests from Australia, Hamm (see 20, 118) reported in 1920 on the insects attacking this weed in England. Australian work was begun in 1928 by Garthside (20). Efforts have continued to the present time. A thorough study was made by Wilson in France from 1935 to 1940 (118). In the early 1950's further attempts were made to establish the gall fly, *Zeuxidiplosis giardi* (Kieffer).

Wilson (118) listed 37 species of *Hypericum*-feeding insects highly restricted in diet. Many of these were released in Australia from 1929 to 1939, but only three became established: a buprestid, *Agrilus hyperici* (Creutzer), and two chrysomelids, *Chrysolina quadrigemina* (Suffrian) and *Chrysolina*

hyperici (Forster) (=*Chrysomela hyperici* Forster). These insects have controlled the weed in certain open lands of Victoria, and, more recently, in Western Australia, (14 15, 64, 81, 124), but effort to obtain more inclusive control by establishment of additional species is continuing.

United States.—It has been estimated that just previous to effects from biological control of St. Johnswort in the western United States, this weed infested the following acreages: 2,333,000 in California (91), 1,250,000 in Oregon (40), 1,000,000 in Idaho,[3] 200,000 in Washington (60), and 200,000 in Montana (71). Morris & Meuchel (71) stated that it could conceivably infest 20,000,000 acres in Montana alone if uncontrolled, as it was spreading rapidly. The total was about 5,000,000 acres, much of it heavily infested and the land almost useless.

In 1943 the late H. S. Smith of the University of California initiated biological control of this weed. With the co-operation of C. P. Clausen, then head of the Division of Foreign Parasite Introductions of the United States Department of Agriculture, the permission to import *Hypericum*-feeding insects was obtained. J. K. Holloway and Huffaker were assigned the problem in California.

Five species of insects were released, and four became established. In addition to the three established in Australia, the gall fly, *Z. giardi,* is well established but of little practical importance [Holloway & Huffaker (43, 44)].

The efforts in California have been a complete success. Largely because of the action of *C. quadrigemina,* the weed has been reduced to less than 1 per cent of its former abundance and no longer constitutes any problem whatever [Huffaker & Kennett (54)]. Variable success has also been obtained in Oregon, Washington (60), Idaho (110), and Montana (93). As in Australia, infestations in colder or shadier locations and those on gravelly soils have not come under the excellent control of those on good sites in the open sun.

New Zealand.—Simultaneously with the work in California, New Zealand entered the program of biological control of St. Johnswort. *C. hyperici,* introduced via Australia, has, according to Miller (67), given outstanding control over extensive areas of St. Johnswort, and of the congener *Hypericum androsaemum* Linnaeus, to some degree (68).

Other countries.—The governments of Canada and Chile also introduced insects for control of this weed. The progress of the work in Canada has been closely studied by Smith (101), who also has just reported (100) the introduction in Canada of *Chrysolina varians* Schaller from Sweden. It is too early to judge what the final results may be, although the control

[3] The writer increased the figure of 600,000 as reported by Tisdale *et al.* (110) to 1,000,000 because of those authors' statement: ". . . but there is considerable evidence that the area is actually much greater," and because the higher figure seems more accurate, comparing with the areas in Oregon and California.

achieved by *C. quadrigemina* and *C. hyperici* in Canada has been disappointing.

Koster's Curse, Clidemia hirta D. Don

Fiji.—The shrub *Clidemia hirta,* which is not browsed by stock, became established in Fiji prior to 1890. By 1919 thousands of acres were infested. Pastures and coconut and rubber plantations suffered heavy losses. As with *Lantana,* its seeds were spread readily by the mynah bird.

A study of the natural checks on *C. hirta* in Trinidad was begun in 1920. Further impetus was given the work through Taylor's visit to Trinidad in 1927, and the work of Cook, which showed the thrips, *Liothrips urichi* Karny, to be very restricted in its diet (97). Simmonds then studied its insect enemies in Trinidad, many of which seemed effective (97).

Since only *L. urichi* proved safe, it was the sole species introduced, although no great hope was held for its success. Releases were made in 1930. Within 18 months it spread readily across several miles of sea and jungle. In 1933, Simmonds (97) stated, ". . . the thrips can be definitely said to have brought the weed under control over large areas, not by directly killing it, but by so inhibiting its growth that it was no longer able to compete with the surrounding vegetation" [*Mimosa* and Para grass on paddocks, and *Micania* on coconut estates, for example]. Paine (78) reported success on Taveuni generally; yet, his experiments in upland country showed a striking contrast when cattle were withheld from the thick "Curse" areas and when they were permitted to graze. The cattle prevented the competing plants, *Micania,* Thurston grass and Para grass, from smothering the weed.

Hawaii.—During the past few years the thrips *L. urichi* was introduced also into Hawaii and established via Fiji, and Fullaway (37) stated in 1958 that it ". . . caused defoliation but seems to flourish best in sunny spots and doesn't do so well in the shade."

Black Sage, Cordia macrostachya (Jacquin) Roemer & Schultes

Mauritius.—This shrub was accidentally introduced in Mauritius about 1890. It forms a dense scrub and became a major pest on grazing lands and sugar-cane and hemp plantations.

Biological control was initially contemplated in 1939, and preliminary work was started in Trinidad in 1944 [Williams (116)]. The first insects studied were leaf beetles, *Physonota* and *Schematiza.* Subsequent to conduction of feeding tests, *Physonota alutacea* Boheman was introduced. After additional specificity tests in Mauritius, it was released in 1947 and 1948, but failed to establish. The failure was shown experimentally to result in part, at least, from the action of ants (115). *Schematiza cordiae* Barber was imported in 1947 and, passing the required tests, was released in 1948. By 1950 much of the scrub had succumbed to the beetle's attack and its aggressiveness elsewhere was greatly reduced (2). A third insect, the seed-infesting *Eurytoma* sp., was introduced in 1949 and immediately became an

important factor in the control of *C. macrostachya,* for by 1951 it was destroying about 50 per cent of the seeds near the liberation sites (2).

Williams (116) commented, "The multiplication of *Schematiza* was so rapid that ascendency over the plant was achieved after the passage of one complete summer of unchecked activity." In a report in 1954 (2) it was stated,

> It is considered that the present very satisfactory degree of control will at least be maintained owing (*a*) to the reduced competitive power of *Cordia* consequent upon the presence of *Schematiza,* and (*b*) to reduced rate of reproduction of the plant which results from both vegetative suppression and direct seed attack by *Eurytoma.*

Pamakani or Crofton Weed, Eupatorium adenophorum Sprengel

This shrub is a native of tropical America and is known as pamakani in Hawaii and Crofton weed in Australia. It spreads rapidly by means of buoyant seeds and forms thickets up to 10 feet in height, crowding out forage species (46).

Hawaii.—Eupatorium adenophorum was introduced in Hawaii about 1900, presumably for medicinal purposes. It occurs from sea level to about 7000 ft. in a variety of climatic zones, but prefers moist situations (46). Estimates have it as rendering useless about 25,000 acres of grazing lands (35). It can be controlled by conventional means, but this may be unfeasible (46).

In 1945 a gall fly, *Procecidochares utilis* Stone, was introduced from Mexico and within a few years gave effective control. Fullaway (37) stated that it ". . . put thousands of acres, formerly of no use on account of thick pamakani, into good grass forage." (See also 5.)

Australia.—P. utilis was also introduced in 1951 and 1952 in both Queensland and New South Wales, where *E. adenophorum* is regarded as a very serious threat, and, previous to this introduction, was spreading rapidly in pasture lands [Wilson (121, 123, 124)]. Wilson (124) added that the insect now promises a contribution to a solution. In event that control is inadequate, Krauss has suggested several promising Mexican insects (see 123).

Manuka Weed, Leptospermum scoparium Forster

New Zealand.—Manuka, although a native, is a harmful weed in New Zealand on rather low-value ranges. But since it is said to have value in preventing erosion, strongly differing opinions prevail as to the desirability of its biological control.

In the early 1940's it was noted that a mealybug, *Eriococcus* sp., now known to be the Australian *E. orariensis* Hoy, was killing the scrub. Its spread was fostered (47, 95), and now, wrote Miller (67) it ". . . has literally wiped out thousands of acres of the scrub which is an agricultural weed." Thus, an accidental introduction was converted to a deliberate and successful biological control of a *native* plant. An expected erosion problem resulting from destruction of Manuka has not materialized [Sewell (95)].

Gorse, Ulex europaeus Linnaeus

This spiny leguminous shrub, indigenous in western Europe, is considered a noxious weed in New Zealand (23), Australia (104), Tasmania (109), Hawaii (46), and the United States (45, 92). It forms almost impenetrable thickets on grazing lands.

New Zealand.—Gorse has been a pest in New Zealand since 1900, but in some parts of the country the young plants are grazed by sheep (23). Consequently, attempts at biological control were aimed only at controlling its seeding and thus its spread (66). Its insect enemies and general ecology in England were investigated by Chater (13). After further studies in England in the late 1920's (23), a seed weevil, *Apion ulicis* Forster, was introduced in 1936. Miller (67) reported that the main crop of seeds is destroyed up to 98.67 per cent, but that the 1.33 per cent not destroyed, together with the "off-crop" which largly escapes destruction, is ". . . quite sufficient to allow the gorse to spread." One wonders if it spreads as fast as before.

Hawaii.—In recent years gorse has formed extensive thickets on range lands in Hawaii. *A. ulicis* was introduced via New Zealand in 1949 to curtail its spread (46). The degree of success has not been reported. About five years later, French stock of this weevil was supplied by H. L. Parker, and it appears superior to the earlier stock. Also, in 1957 other gorse insects were obtained from France through Parker and from England through D. J. Clark but in neither instance was sufficient material emerged or brought out of diapause for release [Davis (24)]. Davis is beginning new explorations for gorse insects in Europe and North Africa.

United States.—Gorse is a relative newcomer along the Pacific Coast in California, Washington, and Oregon, where it invades open ranges. The infested area in California in 1951 was estimated at 15,000 acres (92). This is only a trace of its vast potential in this region. Biological control was initiated in 1948, and *A. ulicis* was released in 1952. Although it is well established in California and Oregon, its ability to curtail spread can only be inferred from the extensive destruction of seeds [Holloway & Huffaker (45)]. This weevil cannot destroy existing stands, but a renewed program for introduction of insects which may do so is contemplated.

Australia.—The gorse weevil was also established on Tasmania in 1939 (124). No report concerning its destruction of gorse seeds has been seen.

Tansy Ragwort, Senecio jacobaea Linnaeus

This composite weed is highly toxic to cattle and horses; it causes cirrhosis of the liver, and, at sublethal levels, markedly reduces butterfat production of dairy cows. Miller (66) related an example of a farmer whose annual butterfat production was reduced within three years to only 10 per cent of the previous level.

New Zealand.—The cinnabar moth, *Tyria jacobaeae* Linnaeus, was known to be destructive to this plant in England, and, in 1927 efforts were initiated to establish it in New Zealand. Initial results were promising,

but near annhilation of the insects in the field followed (66). Miller recently reported (67) that ". . . [*T. jacobaeae*] was reduced to ineffectiveness by birds and especially by parasites of its near relative, *Nyctemera annulata* Boisduval, which itself can temporarily play havoc with the weed on very rare occasions." He stated that *T. jacobaeae* still occurs rarely, but not in effective numbers.

A seed fly, *Pegohylemyia jacobaeae* Meade, was also introduced. Miller reported (67) that with this species also ". . . establishment was excellent at first, and the insect yet occurs in certain biological areas where a survey showed that it exercised a minor degree of control of the ragwort seeds." In fact, his report for 1953 and 1954 credited *P. jacobaeae* with infesting up to 98 per cent of the flowers in 1954 (69).

Australia.—Since ragwort is a pest of importance in Victoria, biological control efforts were made at intervals from 1930 to 1937 (12). *T. jacobaeae* was first introduced via New Zealand and later from England. Although liberated at a number of apparently favorable places, they failed to establish, reportedly because of attack by native predators [Currie & Fyfe (19)]. The seed fly, *P. jacobaeae,* was also introduced in 1938 and 1939, but the work was discontinued pending developments in New Zealand (12).

Emex, Emex spinosa Campdera and Emex australis Steinheil

Hawaii.—Emex is a serious weed on range lands and in cultivated crops in Hawaii. It takes hold especially in bare spots and encroaches on forage species. It is a particular nuisance because of its spiny burs. While the acreage infested is on record as being only a hundred acres or more, in the last 10 years *Emex* has spread extensively and at an alarming rate (46).

Biological control was begun in 1950 in Italy and Sicily. *Lixus algirus* Linnaeus was found to attack the broad bean as well as *Emex* and therefore could not be introduced. Krauss renewed the work in 1956 and sent from Africa three species; two, not yet identified, were not released, but the third, a weevil, *Apion antiquum* Gyllenhal, was released and shows some promise. Later, C. J. Davis sent from Morocco a stem-boring weevil, *Apion neofallax* Warner, which has not yet been released [Fullaway (34)].

Piri-Piri or Bidi-Bidi, Acaena sanguisorbae Vahl

New Zealand.—It is the burs of *A. sanguisorbae* that account for the weed's great harmfulness. Miller (66) reported in 1936 that *A. sanguisorbae* was responsible for reducing the annual wool crop in New Zealand by about £250,000. In the late 1920's biological control, perhaps the first of its kind against a native weed, was initiated with a study of the insects attacking *Acaena* in Chile.

The flea-beetle, *Haltica virescens* Blanchard, in Chile and another, *Haltica pagana* Blackburn, the latter reportedly somewhat effective in Australia (104), could not be introduced into New Zealand because neither

satisfied the strict host requirements and *Acaena* is a member of the exceedingly important Rosaceae. However, the Chilean sawfly, *Antholcus varinervis* (Spinola), was tested and later released. Contrary to the statement of Sweetman (104, p. 379) in 1936 that it ". . . has produced excellent results in controlling the pest," this insect failed to establish, although it appeared to do well in certain localities at times. Miller (67) has this to say in 1958: "It is quite possible that birds are responsible for the failure in the field, because at the time when sawfly larvae first emerge in the late winter and early spring, insect food is not particularly prevalent. . . ."

Noogoora Bur, Xanthium strumarium Linnaeus (= X. pungens Wallroth)

Australia.—Noogoora bur is a damaging weed on grazing lands in Queensland and New South Wales. Its burs adhere to the wool of sheep, greatly lowering its value. The lands are too low in value to permit employment of chemicals for control.

Australian workers (12) found many insects attacking the group (cockleburs) in the United States, and a lesser number in India. Two cerambycids, *Nupserha antennata* Gahan in India and *Mecas saturnina* Le Conte in the United States, showed promise but were not introduced because they developed on certain other composites, mainly wild sunflowers and Jerusalem artichoke.

The American seed fly *Euaresta aequalis* Loew was released in the early 1940's (12). Although it is established, results have been discouraging. Nearly 100 per cent of the seeds must be destroyed for effectiveness. Also, erratic germination of *Xanthium* in certain areas, leading to irregular presence of the burs from year to year, prevents the insect's survival in those areas (12). Wilson (121) commented on the cerambycids, "The problem has arisen, therefore, whether the importance of attempting to control Noogoora bur should over-ride the risks involved to a limited number of composites." Dodd (28) feels that further study of these borers is warranted and that should such work fail to show a greater risk than is already known, their introduction should be permitted. Wilson (124) has just reported reopening of studies to answer these questions.

Nutgrass, Cyperus rotundus Linnaeus

Nutgrass, a perennial sedge, is a pest in temperate and tropical countries. Because of its underground bulbs, it is difficult to control by conventional means.

Hawaii.—In Hawaii nutgrass invades sugar-cane plantations, gardens, and lawns. Three insects were studied as a possible solution to the problem. Two from the Philippines, a bulb borer, *Bactra truculenta* Meyrick, and a weevil, *Athesapeuta cyperi* Marshall, were released in 1925 [Fullaway (35)]. Both species became established. Williams (114) stated that *B. truculenta* sometimes does effective work locally, adding that the severest

check on it is the egg parasite, *Trichogramma minutum* Riley. The weevil is considered of even less effect.

Australia.—Nutgrass is also a pest of cultivated fields in Australia where crop rotation and temporary soil sterilization have given some relief. *B. truculenta* and *A. cyperi* were tested from 1937 to 1940, but were not released because they fed on related species of *Cyperus* which are of some value on certain pastures [Cashmore & Campbell (12)].

SOME CURRENTLY CONSIDERED EFFORTS

A few weeds which have demanded some attention or may soon do so will be discussed briefly.

Halogeton glomeratus C. A. Mey, a very important toxic weed in western United States, has been actively studied in the Mediterranean region and the Near East by the United States Department of Agriculture, but as yet no insects suitable for introduction have been found.

Cytisus scoparius Link, or Scotch broom, is currently being investigated as a subject for biological control in the western United States. Some insects of promise are indicated [Parker (80)].

Acanthospermum hispidum De Candolle is a pest in parts of Nigeria, West Africa. Lloyd (61) recently reported that its insect enemies in Brazil and Argentina appear ineffective. He found the weed scarce in Colombia and Venezuela, but the brief study there turned up no promising insects.

Heliotropium europaeum Linnaeus, a prolific weed in Australia, was investigated by Wilson (123) in the Mediterranean area in 1950. He concluded that of the insects observed some were polyphagous and others rather ineffective.

Myrica faya Aiton, or firebush, forms thickets and encroaches on open pastures in Hawaii, particularly where the rainfall is 35 in. or more a year (46). N. L. H. Krauss sent an olethreutid, *Strepsicrates smithiana* (Walsingham), in 1955 and 1956, and it was released [Weber (113)].

Melastoma malabathricum Linnaeus is a slow spreading pest shrub on grazing lands in Hawaii, where it crowds out forage species and forms thickets. It has no forage value (46). An unidentified tortricid from the Philippines has recently been cleared for release against this weed [Davis (24)].

Stachytarpheta jamaicensis Vahl, or blue rat tail, is a major weed of poor grazing lands in parts of Fiji, counts having established its incidence in certain areas as from 10 to 25 per cent of the cover. It is also a major pest in Samoa, Tonga, and parts of the Solomon Islands (98). Simmonds (98) reported destruction of its seeds by a cecidomyiid in Trinidad and suggested biological control. Apparently little has been done.

Other weeds which are active subjects or being considered in North America include *Tribulus terrestris* Linnaeus (puncture vine), *Centaurea* spp. (star thistles and knapweed), *Senecio jacobaea* Linnaeus (tansy ragwort), *Salvia aethiopis* Linnaeus (Mediterranean sage), *Linaria dalmatica*

Miller (Dalmatian toadflax), and *Linaria vulgaris* Miller (toadflax), for example.

CONCLUSION

Biological control of weeds by insects can be highly rewarding, as demonstrated by the number of partial or complete successes on record. In most instances, the initial results have been indicative of a permanent control. However, in some cases, the striking initial effects have not been maintained, partly because of an increase in the influence of natural enemies of the introduced control agents. It is clear that the gains to be won far exceed the element of risk involved. This method of weed control is soundly based in the science of ecology and has contributed to that field by demonstration of the tremendous role insects play in helping to determine the plant composition of the earth. Biological control of weeds is in its infancy, and the early feeling that it should be used only as a last resort is, where proper precautions are taken, unfounded.

LITERATURE CITED

1. Andrewartha, H. G., and Birch, L. C., *The Distribution and Abundance of Animals* (University of Chicago Press, Chicago, Ill., 782 pp., 1954)
2. Anonymous, *Rept. Commonwealth Entomol. Conf., 6th Meeting,* 281–84 (London, England, 1954)
3. Arnott, D. A., *Proc. Entomol. Soc. Brit. Columbia,* **53,** 14–5 (1957)
4. Bess, H. A. (Private communication, 1957)
5. Bess, H. A., and Haramoto, F. H., *Proc. Intern. Congr. Entomol., 10th Meeting* (Montreal, Que., Canada, 1956), **4,** 543–48 (1958)
6. Bews, J. W., *Ann. Natal Museum,* **4,** 367–569 (1920)
7. Bodenheimer, F. S., *Z. angew. Entomol.,* **16,** 433–50 (1930)
8. Brues, C. T., *Insect Dietary* (Harvard University Press, Cambridge, Mass., 466 pp., 1946)
9. Brues, C. T., *Insects, The Yearbook of Agriculture (U. S. Dept. Agr.),* 37–42 (1952)
10. Bugbee, R. E., and Reigel, A., *Am. Midland Naturalist,* **33,** 117–27 (1945)
11. Cameron, E., *J. Ecol.,* **23,** 265–322 (1935)
12. Cashmore, A. B., and Campbell, T. G., *J. Council Sci. Ind. Research,* **19,** 16–31 (1946)
13. Chater, E. H., *Bull. Entomol. Research,* **22,** 225–35 (1931)
14. Clark, L. R., *Australian J. Zool.,* **1,** 1–69 (1953)
15. Clark, L. R., and Clark, N., *Australian J. Agr. Research,* **3,** 29–59 (1952)
16. Cole, L. C., *Ecology,* **36,** 538–39 (1955)
17. Cook, C. W., *Ecology,* **23,** 209–14 (1942)
18. Currie, G. A., *Imperial Bur. Pastures Forage Crops, Herbage Publication Series Bull., No. 27,* 113–30 (1940)
19. Currie, G. A., and Fyfe, R. V., *J. Council Sci. Ind. Research,* **11,** 289–301 (1938)
20. Currie, G. A., and Garthside, S., *Council Sci. Ind. Research Pamphlet, No. 29,* 28 pp. (1932)
21. Dameron, W. H., and Smith, H. P., *Texas Agr. Expt. Sta. Bull., No. 575,* 55 pp. (1939)

22. David, A. L., and Muthukrishnan, T. S., *Indian J. Entomol.*, **15–16**(3), 219–24 (1953)
23. Davies, W. M., *Ann. Appl. Biol.*, **15**, 263–85 (1928)
24. Davis, C. J. (Private communication, 1958)
25. Davis, C. J., *Proc. Hawaiian Entomol. Soc.*, **16**, 356–58 (1958)
26. Dethier, V. G., *Chemical Insect Attractants and Repellents* (The Blakiston Co., Philadelphia, Pa., 289 pp., 1947)
27. Dethier, V. G., *Evolution*, **8**, 33–54 (1954)
28. Dodd, A. P., *Biological Control of Weeds*, 121–23 (Weed Control Conf., Roseworthy Agr. Col., Roseworthy, S. Australia, Session 5, August, 1954)
29. Dodd, A. P., *The Biological Campaign Against Prickly Pear* (Commonwealth Prickly Pear Board, Brisbane, Australia, 177 pp., 1940)
30. Dodd, A. P., *Minutes Entomol. Soc. Queensland* (Brisbane, Queensland, November, 1957)
31. Dodd, A. P., *Proc. Hawaiian Entomol. Soc.*, **15**, 41–44 (1953)
32. Essig, E. O., *J. Econ. Entomol.*, **41**, 58–61 (1948)
33. Flanders, S. E., *Proc. Intern. Congr. Entomol., 10th Meeting* (Montreal, Que., Canada, 1956), **4**, 541 (1958)
34. Fullaway, D. T., *Control of the Pasture Weed*, Emex spinosa (In press)
35. Fullaway, D. T., *Fifty Years Progress in the Biological Control of Weeds—A Review* (Mimeographed report, ca. 1954)
36. Fullaway, D. T., *J. Econ. Entomol.*, **47**, 696–700 (1954)
37. Fullaway, D. T. (Private communication, 1958)
38. Gardner, J. C. M., *Indian Forester*, **70**, 139–40 (1944)
39. Gardner, T. R. (Private communication, 1958)
40. Harris, G. A., *Northern Rocky Mountain Forest and Range Expt. Sta. Paper, 26* (1951)
41. Heinze, K., *Z. Pflanzenkrankh. u. Pflanzenschutz*, **63**, 689–93 (1956)
42. Holloway, J. K. *Proc. Western Weed Control Conf., 14th Meeting*, 59–62 (Tucson, Ariz., February, 1954)
43. Holloway, J. K., and Huffaker, C. B., *Insects, The Yearbook of Agriculture (U. S. Dept. Agr.)*, 135–40 (1952)
44. Holloway, J. K., and Huffaker, C. B., *J. Econ. Entomol.*, **46**, 65–67 (1953)
45. Holloway, J. K., and Huffaker, C. B., *J. Econ. Entomol.*, **50**, 498–99 (1957)
46. Hosaka, E. Y., and Thistle, A., *Univ. Hawaii Exten. Bull. No. 62*, 39 pp. (1954)
47. Hoy, J. M., *New Zealand J. Agr.*, **79**, 321–24 (1949)
48. Huffaker, C. B., *Calif. Dept. Agr. Bull.*, **40**, 241 (1952)
49. Huffaker, C. B., *Hilgardia*, **27**, 101–57 (1957)
50. Huffaker, C. B., *Proc. Intern. Congr. Entomol., 10th Meeting* (Montreal, Que., Canada, 1956), **2**, 625–36 (1958)
51. Huffaker, C. B., *Proc. Intern. Congr. Entomol., 10th Meeting* (1956), **4**, 533–42 (1958)
52. Huffaker, C. B., and Holloway, J. K., *Ecology*, **30**, 167–75 (1949)
53. Huffaker, C. B., and Kennett, C. E., *Hilgardia*, **26**, 191–222 (1956)
54. Huffaker, C. B., and Kennett, C. E., *J. Range Management* (In press)
55. Huxley, J. S., in *Evolution as a Process*, 1–23 (George Allen and Unwin Ltd., London, England, 367 pp., 1954)
56. Imms, A. D., *Recent Advances in Entomology*, 410–19 (The Blakiston Co., Philadelphia, Pa., 425 pp., 1937)

57. Imms, A. D., *Trans. Intern. Congr. Entomol., 4th Meeting (Ithaca, N.Y., 1928)*, **2**, 10–17 (1930)
58. Janvier, H., *Ann. sci. nat., Zool.*, Ser. 10, No. 16, 209–56 (1933)
59. Krauss, N. L. H., *Proc. Hawaiian Entomol. Soc.*, **15**, 123–25 (1953)
60. Lange, A. W., *Weeds*, **6**, 68–70 (1958)
61. Lloyd, D. C., *Can. Entomologist*, **88**, 613–22 (1956)
62. Louis-Marie, Pére, *Rev. Oka Agron. méd. vet.*, **31**, 138–44 (1957)
63. Maehler, K. L., *J. Econ. Entomol.*, **41**, 991–92 (1948)
64. Meadley, G. R. W., *West. Australia Dept. Agr. J.* [3] **5**, 661–64 (1956)
65. Miller, D., *Imperial Bur. Pastures Forage Crops, Herbage Publications Series, Bull. No. 27*, 153–57 (1940)
66. Miller D., *New Zealand J. Sci. and Technology*, **18**, 581–84 (1936)
67. Miller, D. (*Private communication*, 1958)
68. Miller, D., *Rept. of the Director, 1948–49, Cawthron Institute*, 39–44 (Nelson, New Zealand, 1949)
69. Miller, D., *Rept. of the Director, 1953–54, Cawthron Institute*, 39 (Nelson, New Zealand, 1954)
70. Milne, A., *Can. Entomologist*, **89**, 193–213 (1957)
71. Morris, M., and Meuchel, J., *Montana Forest and Conserv. Expt. Sta. Bull.*, **4**, 8 pp. (1956)
72. Muir, F., *Proc. Hawaiian Entomol. Soc.*, **3**, 28–42 (1914)
73. Narayanan, E. S., *Rept. Commonwealth Entomol. Conf., 6th Meeting*, 39 (London, England, 1954)
74. Naude, T. J., *World Crops* **8**, 401–02 (1956)
75. Nicholson, A. J., *Ann. Rev. Entomol.*, **3**, 107–35 (1958)
76. Nicholson, A. J., *Australian J. Zool.*, **2**, 9–65 (1954)
77. Osborn, H. T., *Hawaiian Planters' Record*, **28**, 546–59 (1924)
78. Paine, R. W., *Agr. J. Colony Fiji Dept. Agr.*, **7**, 10–21 (1934)
79. Parham, J. W., Mune, T. L., and O'Connor, B. A., *Agr. J. Colony Fiji Dept. Agr.*, **27**, 28–32 (1956)
80. Parker, H. L. (Private communication, 1958)
81. Parsons, W. T., *A Review of the Control of St. John's Wort in Victoria*, 121–23 (Weed Control Conf., Roseworthy Agr. Col., Roseworthy, S. Australia, Session 5, August, 1954)
82. Pedersen, M. W., *J. Am. Soc. Agron.*, **34**, 769–70 (1942)
83. Perkins, R. C. L., and Swezey, O. H., *Hawaiian Sugar Planters' Assoc. Expt. Sta., Entomol. Series, Bull., No. 16*, 83 pp. (1924)
84. Pettey, F. W., *S. Africa Dept. Agr. Sci. Bull., No. 271*, 163 pp. (1948)
85. Pettey, F. W., *S. Africa Dept. Agr. Bull. No. 296*, 34 pp. (1950)
86. Pettey, F. W., *S. Africa Dept. Agr. Sci. Bull., No. 340*, 36 pp. (1953)
87. Pettey, F. W., and Marais, S. J. S., *S. Africa Dept. Agr. Bull., No. 296*, 34 pp. (1950)
88. Phillips, J., *J. Ecol.*, **19**, 1–24 (1931)
89. Phillips, J. *J. Ecol.*, **23**, 210–46 (1935)
90. Pringle, W. L., *A New Look at Sagebrush Control* (Presented at the annual meeting of the Pacific Northwest Sect., Am. Soc. Range Management, Yakima, Washington, U.S.A., 1955)
91. Pryor, M. R., *Calif. Dept. Agr. Bull.*, **41**, 298 (1953)
92. Pryor, M. R., and Dana, R. H., *Calif. Dept. Agr. Bull., No. 41*, 43–45 (1952)
93. Roemhild, G., *Status of Chrysolina Beetle Project* (Typed report, 1956)

94. Sellers, W. F., *Bull. Entomol. Research,* **43,** 43–50 (1952)
95. Sewell, T. G., *New Zealand J. Agr.,* **79,** 101–4 (1949)
96. Simmonds, F. J., *Bull. Entomol. Research,* **39,** 217–20 (1948)
97. Simmonds, H. W., *Bull. Entomol. Research,* **24,** 345–48 (1933)
98. Simmonds, H. W., *Agr. J. Colony Fiji Dept. Agr.,* **7,** 3–10 (1934)
99. Smith, H. S., *J. Econ. Entomol.,* **28,** 873–98 (1935)
100. Smith, J. M. (*Private communication,* 1958)
101. Smith, J. M., *Proc. Intern. Congr. Entomol., 10th Meeting* (Montreal, Que., Canada, 1956), **4,** 561–65 (1958)
102. Solomon, M. E., *Ann. Rev. Entomol.,* **2,** 121–42 (1957)
103. Steiner, L. E., *Proc. Hawaiian Entomol. Soc.,* **15,** 291 (1954)
104. Sweetman, H. L., *The Biological Control of Insects, With a Chapter on Weed Control* (Comstock Publishing Associates, Ithaca, N.Y. 461 pp., 1936)
105. Thompson, W. R., *Ann. Rev. Entomol.,* **1,** 379–402 (1956)
106. Thompson, W. R., *Parasitology,* **21,** 269-81 (1929)
107. Thompson, W. R., *Rept.. Commonwealth Entomol. Conf., 6th Meeting,* 89–95 (London, England, 1954)
108. Tillyard, R. J., *Proc. Royal Soc. Tasmania,* 51–86 (1929)
109. Tillyard, R. J., *Trans. Intern. Congr. Entomol., 4th Meeting (Ithaca, N.Y., 1928),* **2,** 4–9 (1930)
110. Tisdale, E. W., Pringle, W. L., and Hironaka, M., *Univ. Idaho Forest, Wildlife and Range Expt. Sta., Research Note, 6,* 9 pp. (1953)
111. Tisdale, E. W., and Zappetini, G., *J. Range Management, 6,* 225–36 (1953)
112. Van der Goot, P., *Overdruk uit Landbouw,* **16,** 413–29 (1940)
113. Weber, P. W., *Proc. Hawaiian Entomol. Soc.,* **16,** 313–15 (1957)
114. Williams, F. X., *Handbook of the Insects and Other Invertebrates of Hawaiian Sugar Cane Fields* (Hawaiian Sugar Planters' Assoc., Honolulu, 400 pp., 1931)
115. Williams ,J. R., *Bull. Entomol. Research,* **40,** 479–80 (1950)
116. Williams, J. R., *Bull. Entomol. Research,* **42,** 455–63 (1951)
117. Williams, J. R., *Rept. Commonwealth Entomol. Conf., 6th Meeting,* 95–98 (London, England, 1954)
118. Wilson, F., *Council Sci. Ind. Research Bull., No. 169,* 87 pp. (1943)
119. Wilson, F., *Intern. Union Biol. Sci.,* [B], No. *5,* 53–64 (1949)
120. Wilson, F., *New Biology,* No. 8, 51–74 (Penguin Books, Inc., Baltimore, Md., 1950)
121. Wilson, F., *Proc. Intern. Grassland Congr., 6th Meeting,* **6,** 567–72 (University Park, Pa., 1952)
122. Wilson, F., *Proc. Pacific Sci. Congr., Roy. Soc. New Zealand, Wellington,* **3,** 294–99 (Wellington, New Zealand, 1953)
123. Wilson, F., *Some Aspects of the Biological Control of Weeds in Australia,* 105–13 (Weed Control Conf., Roseworthy Agr. Col., Roseworthy, S. Australia, Session 5, August, 1954)
124. Wilson, F., *The Results of Biological Control Investigations in Australia and New Guinea* (In press)

MICROBIAL CONTROL OF INSECT PESTS[1]

By Y. Tanada
Laboratory of Insect Pathology, Department of Biological Control, University of California, Berkeley, California

Experimentation during the past decade has proved that microbial control, or the use of pathogenic microorganisms, is an integral part of applied insect control. Microbial control is of special value when other methods have been found inadequate, for example, when insecticidal residues are toxic to man, insect parasites, and predators. Although some insect pathogens may replace certain insecticides, parasites, and predators, most of the successful applications of pathogens in the future will probably be made in conjunction with these other agents of control.

Microbial control has advanced rapidly by adopting many of the principles and methods used in chemical control and in the parasite-predator branch of biological control. Thus microbial control is closely interrelated with these two fields. Steinhaus (163) has pointed out that if the "economic level" of the host density is considerably lower than the "threshold level" of the disease, control will be temporary and the pathogens have to be applied repeatedly, as with chemical insecticides. This has been the case when microbial control has been applied against certain insects on field crops. If the economic level of the host density is higher than the threshold level of the disease, then control can be just as permanent as that accomplished by insect parasites. Such permanent control has been obtained in the case of certain insects of forest, turf, and pasture (23, 26, 80, 83, 219).

Microbial control has been applied in nearly all of the major countries of the world. Canada, Czechoslovakia, Germany, Japan, Switzerland, and Yugoslavia have contributed mainly to forest insect control; Australia, Egypt, England, Indonesia, and New Zealand to field insect control; France, Russia, and the United States to both forest and field insect control. Most of the field tests have been conducted with viruses and bacteria. Rickettsiae, fungi, protozoa, and nematodes have not been extensively utilized, mainly because of difficulties inherent in their use or because of their extreme dependence on environmental conditions.

Inasmuch as Steinhaus (161, 165) has thoroughly covered the historical aspects as well as the advances in microbial control up to 1949, the present review is restricted largely to literature appearing since that time. Recently two excellent reviews on insect pathology have appeared: the first by Steinhaus (166) on the general and applied aspects of insect pathology, and the other by Bergold (15) on insect viruses and their application in insect control. The reader is encouraged to consult both of them, especially on cer-

[1] The survey of the literature pertaining to this review was completed in April, 1958.

tain aspects of insect pathology which are not covered by the present review. Other publications have appeared on the general aspects (34, 38, 72) and the potentialities (164) of microbial control, and on the role of insect pathogens, such as fungi (9, 9a), viruses (14, 64, 65, 159), protozoa (212), and nematodes (218) in insect control.

Because of space limitations, the examples of microbial control will be restricted almost entirely to those effected by man, although there are numerous examples of the importance of pathogenic microorganisms in natural control [see Steinhaus (166); Bergold (15)]. The production of pathogens [see Steinhaus (164)], the pathogenicity of insect pathogens to plants and higher animals [see Steinhaus (169)], and host specificity [see Bergold (15) for viruses] will not be considered.

Use of Pathogens

The use of viruses.—Of the five general groups of virus diseases—nuclear and cytoplasmic polyhedroses, granuloses, polymorphic inclusion viroses, and the noninclusion viroses—only the viruses of the granuloses and nuclear polyhedroses have been utilized extensively in microbial control. Following the outstanding natural control of the European spruce sawfly, *Diprion hercyniae* (Hartig), by an accidentally introduced polyhedrosis virus, the Canadian investigators, in 1949, brought in from Europe a polyhedrosis virus of the European pine sawfly, *Neodiprion sertifer* (Geoffroy) [Bird (23)]. When sprayed on pine forests, this virus has been so effective that, in general, it has replaced the use of insecticides [Bird (26)]. The virus has also been applied successfully in the United States [Dowden & Girth (49); Benjamin *et al.* (11)] and even in its native home in Europe, where, although it is a major natural mortality factor of the pine sawfly [Breny (33); Franz & Niklas (67)], spray application has caused a faster reduction in the sawfly population than the natural spread of the virus [Franz & Niklas (67)]. Similar effective and prolonged results have been obtained with the application of the virus of the European spruce sawfly [Bird (25)]. However, not all sawfly viruses have been effective against their respective hosts. A virus of the jack-pine sawfly, *Neodiprion americanus banksianae* Rohwer, possessed such low virulence that it was impractical for field use [Bird (26)].

Polyhedrosis viruses have been effectively applied against such forest insects as the pine processionary caterpillar, *Thaumetopoea pityocampa* (Denis & Schiffermüller) [Biliotti *et al.* (22)], and the Great Basin tent caterpillar, *Malacosoma fragilis* (Stretch) [Clark & Thompson (45); Clark & Reiner (43)], but were unsuccessful against *Malacosoma disstria* Hübner [Bergold (12)]. In Switzerland, although a granulosis virus is the major mortality factor of the larch bud moth, *Eucosma griseana* (Hübner) [Martignoni (123); Bovey (32)], it apparently has not been effective when applied as a spray during the phase of population decline [Martignoni & Auer (124)].

Polyhedrosis viruses have been successfully applied against such field- and truck-crop insects as the alfalfa caterpillar, *Colias philodice eurytheme* Boisduval [Steinhaus & Thompson (173); Thompson & Steinhaus (194); Thompson (192)], the cabbage looper, *Trichoplusia ni* (Hübner) [Hall (78); McEwen & Hervey (128)], the gamma noctuid, *Plusia gamma* Linnaeus [Vago & Cayrol (207)], and the wattle bagworm, *Kotochalia junodi* (Heylaerts) [Ossowski (137)]. Applications of granulosis viruses have controlled the imported cabbageworm, *Pieris rapae* (Linnaeus) [Tanada (188); Kelsey (101)], and the European cabbageworm, *Pieris brassicae* (Linnaeus) [Biliotti *et al.* (21); Smith & Rivers (159)], but have failed to prevent fruit damage by the red-banded leaf roller, *Argyrotaenia velutinana* (Walker), in spite of nearly 100-per-cent morbidity among the larvae [Glass (70)].

Although some pathogenic rickettsiae have been found in insects, they have not been applied, as yet, in insect control. However, some of them may be important in the natural control of the cockchafers, *Melolontha* spp. [Wille & Martignoni (222); Niklas (132, 133)], and the Japanese beetle, *Popillia japonica* Newman [Dutky & Gooden (52)].

The use of bacteria.—Among the bacteria, the entomogenous sporeformers have received increased interest in recent years because of their great potentiality for insect control. Thus far, they are the only important bacteria that have been effectively applied in the field. These sporeformers may be roughly divided into two general groups: those related to the milky-disease organism, *Bacillus popilliae* Dutky, and those related to *Bacillus cereus* Frankland & Frankland (noncrystal-bearers) and *Bacillus thuringiensis* Berliner (crystal-bearers).

Field experimentations are still being continued with the introduction and colonization of milky-disease organisms, and they have confirmed the early reports on the prolonged control of the Japanese beetle [White & McCabe (219); Polivka (141)]. The milky-disease organisms and related strains are also effective against the European chafer, *Amphimallon majalis* (Razoum) [Tashiro & White (190); Tashiro (189)]. Approximately 150 bacterial strains and some fungi have been isolated from *Melolontha melolontha* (Linnaeus) in Europe, and field applications with some of the unidentified bacteria have resulted in increased mortality of the white grubs [Wikén *et al.* (220)].

Among the sporeformers, the most extensively utilized group includes the crystal-bearing forms such as *B. thuringiensis* and its close relatives, *alesti, sotto,* "Anduze," and others.[2] The crystals are highly toxic to certain insects and appear to be mainly responsible for the pathogenicity of these bacteria (3, 7, 86, 87, 88, 172). *B. thuringiensis* and its close relatives have

[2] Some confusion exists on the taxonomic status of *Bacillus thuringiensis* Berliner, *Bacillus cereus* var. *alesti* Toumanoff & Vago, *Bacillus sotto* Ishiwata, and other closely related forms as to whether they are true species or varieties. Heimpel & Angus (94) have attempted to clarify the taxonomy of these forms.

been tested against over 100 insect species, mainly Lepidoptera, many of which are highly susceptible to the bacteria [Steinhaus (168); Hall & Dunn (82)]. These bacteria have been used successfully against the oak processionary caterpillar, *Thaumetopoea processionea* (Linnaeus) [Grison & Béguin (73)], the pine processionary caterpillar [Grison & Béguin (73)], the fall webworm, *Hyphantria cunea* (Drury) [Vasiljević (209)], the alfalfa caterpillar [Steinhaus (162)], *Colias lesbia* Fabricius [Faldini & Pastrana (56)], the imported cabbageworm [Tanada (188)], the European cabbageworm (20, 108, 111, 201), the diamond back moth, *Plutella maculipennis* (Curtis) [Tanada (188); Oka (136)], the lablab podborer, *Adisura atkinsoni* Moore [Majumder *et al.* (121)], and the hornworms, *Protoparce quinquemaculata* (Haworth) and *Protoparce sexta* (Johanssen) [Rabb *et al.* (143)]. Limited effectiveness has been obtained against the cabbage webworm, *Hellula undalis* (Hübner), and the cabbage looper, possibly because the spore dosage was too low [Tanada (188)]. Contrary to the promising reports of early workers, *B. thuringiensis* has been ineffective against the European corn borer, *Pyrausta nubilalis* (Hübner), because the larva escapes the spore residue on the plant surface by boring into the leaf whorls [McConnell & Cutkomp (127)]. This bacillus is also not recommended for the grapeleaf skeletonizer, *Harrisina brillians* Barnes & McDunnough, of which some of the larvae are definitely resistant [Hall (77)]. A French product, "Sporeine," which very probably contains spores of *B. thuringiensis*, has curbed the development of the Mediterranean flour moth, *Anagasta kühniella* (Zeller), in flour sheds [Jacobs (98)]. This result confirms the early tests performed by Berliner in 1915. On the other hand, coleopterous stored-grain insects are not sufficiently susceptible to *B. thuringiensis* to warrant its use in practical control [Steinhaus & Bell (171); De & Konar (47)].

Some forms of *Bacillus cereus* are apparently pathogenic for certain insects, such as the larch sawfly, *Pristiphora erichsonii* (Hartig) [this is the first record of a strain of *B. cereus* pathogenic for a hymenopteron—Heimpel (91)], and the codling moth, *Carpocapsa pomonella* (Linnaeus) [Phillips *et al.* (140); Stephens (175)]. These strains have not been effective in field tests against the two insects mentioned.

While some nonsporeforming bacteria are highly pathogenic for many insects in the laboratory, they have been applied only to a limited extent in the field, largely because of their high susceptibility to desiccation. One of the few nonsporeformers used in field application is *Pseudomonas aeruginosa* (Schroeter) Migula, which was isolated by Bucher & Stephens (35) from grasshoppers. Stephens (176) has developed a preservative which has been incorporated into a bacterial spray and a bait by Baird (8). Although the spray and bait did not control the grasshoppers, this attempt does indicate the possibility of field application of nonsporeforming bacteria with the use of preservatives.

The use of fungi.—Whereas fungi were widely utilized several decades

ago, they have seen rather limited field application in recent years, apparently because of their great dependence on proper humidity conditions. Evidence has accumulated that at least some of the so-called "friendly fungi" found on scales in Florida are not primary parasites [Fisher *et al.* (61); Fisher (57)]. In their places a chytrid, *Myiophagus* sp., on purple scale, *Lepidosaphes beckii* (Newman); species of *Hirsutella* on scale insects and mites; and an *Entomophthora* sp. on citrus red mite, *Metatetranychus citri* (McGregor), are apparently important mortality factors under certain conditions (57, 58, 59, 61, 129, 130). Thus far no attempt has been made to apply these promising fungi in microbial control.

Five species of fungi have been found parasitizing the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), under natural conditions [Hall & Dunn (80, 81)]. Although Rockwood (145) has concluded that chances of artificially induced epizootics appear less likely with Entomophthoraceae than with the hyphomycetous fungi, Hall & Dunn (80, 83) have successfully disseminated the former fungi by three different methods into fields infested with aphids apparently free of fungus. This successful application may have been possible because of the recent introduction of the aphid into a new area where the fungi were absent or had not attained their saturation points. Shands *et al.* (155) have recovered the fungus, *Acrostalagmus* probably *A. aphidium* Oudemans, after a spray application of fungus spores among a light population of potato aphid, *Macrosiphum solanifolii* (Ashmead).

Among the Fungi Imperfecti, the genus *Beauveria* appears to offer the most promise in microbial control because of its rather high pathogenicity for a large number of hosts.[3] Field applications of *Beauveria* spp. have been effective against *Melolontha* sp. [Schaerffenberg (149)], the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), [Schaerffenberg (150)], and *Aradus cinnamomeus* Panzer [Smirnov (158)]. There is continued interest in the green muscardine fungus, *Metarrhizium anisopliae* (Metchnikoff) Sorokin, notwithstanding some earlier reports discouraging its use against the rhinoceros beetle, *Oryctes rhinoceros* (Linnaeus) [Nirula *et al.* (135); Nirula (134)]. The green muscardine fungus has not been effective against the wireworms, *Agriotes obscurus* (Linnaeus) and *Agriotes sputator* (Linnaeus), probably because of limiting environmental factors [Fox & Jaques (62)].

In Russia, *Aspergillus ripens* (Corda) has been applied for the control of the pentatomid, *Eurygaster integriceps* Puton [Evlakhova (54, 55)]. Other fungi, such as *Beauveria* and *Fusarium*, also appear effective against the pentatomid [Sulzdalskaya (178)]. Successful control of several insects with species of *Cephalosporium* and *Verticillium* has been reported by Shvetsova & Evlakhova (157).

[3] Recently MacLeod (118) has revised the genus *Beauveria* and reduced 14 species to synonymy with *Beauveria bassiana* (Balsamo) and *Beauveria tenella* (Delacroix) Siemaszko.

From their study of the fungus and bacterial pathogens of the larch sawfly, MacLeod & Heimpel (120) have concluded that fungi in general contribute greatly to the total mortality of insects in Canada. They believe that, because of poor sporulation, a saturation point may never be reached with certain entomogenous fungi of more highly specialized parasitism. This indicates that an increase in infection and morbidity might well be expected from the field application of certain fungi.

The use of protozoa.—Theoretically, protozoa offer considerable promise in microbial control because of the large number of parasitic species found on insects. Perhaps the relative difficulties in studying, identifying, and producing protozoa in large quantities account for the fact that few attempts have been made to use them in field control [Steinhaus (166)]. Their application may also be limited because they generally cause chronic rather than acute infections, although many microsporidia possess sufficient virulence to make them effective pathogens. There is little information on the ecology of protozoan diseases, and the role of protozoa in the regulation of insect population density is apparently underrated [Weiser (212)].

Weiser and his colleagues in Czechoslovakia are at present the chief proponents for the use of protozoa in microbial control. Among the protozoa, Weiser (212) has selected four groups which he considers of value in microbial control: Schizogregarina, Coccidia, Haplosporidia, and Microsporidia. He has listed also some protozoa which show definite promise in microbial control. The microsporidian, *Thelohania hyphantriae* Weiser, has been used with encouraging results against *Hyphantria cunea, Euproctis chrysorrhoea* (Linnaeus) [= *Nygmia phaeorrhoea* (Donovan)], and *Malacosoma neustria* Linnaeus [Weiser (214); Weiser & Veber (216, 217)]. A 100-per-cent infection was obtained in each case, but the larvae remained alive from 14 days to about a month. The microsporidian, *Nosema bombycis* Naegeli, which causes the classic pébrine of the silkworm is so highly infectious for the fall webworm that it has been recommended for field use against the insect [Machay (114)]. But since the disease might spread to silkworms under culture, the advisability of using *N. bombycis* in sericultural areas is questionable.

Recent attempts with *Perezia pyraustae* Paillot against the European corn borer [Zimmack *et al.* (224)] and with *Nosema infesta* Hall against the sod webworm, *Crambus bonifatellus* (Hulst) [Hall (76)], have largely failed. Blunck (31), from his extensive observations, has concluded that microsporidia may not be suitable for the biological control of the noxious Pieridae in Europe.

The use of nematodes and algae.—As with the protozoa, very few field attempts have been made with nematodes for insect control. However, nematodes may eventually provide a significant number of effective agents because of the large numbers that are internal parasites of insects.

A very promising nematode of the family Steinernematidae, which is as yet unidentified, has been discovered by Dutky (51) in codling-moth larva.

The nematode carries with it a species of bacterium into the haemocoel of the insect host, and the bacterium not only kills the host but serves as food for the nematode. Under laboratory conditions, a very large number of insects in several different orders have been found susceptible to the nematode and its associated bacterium. These tests have been conducted under moisture conditions suitable for the nematode, but such conditions may not be available under the normal habitat of some of the test insects. This nematode has been used on banded apple trees for the control of codling moth and has caused high mortality among the larvae [Dutky & Hough (53)].

Several species of blue-green algae, when transplanted into rice fields, have shown some promise in the control of mosquito larvae [Gerhardt (68, 69)]. The algae apparently produce substances toxic to the mosquito larvae.

Repellency, Infection, and Transmission

It is generally known that pathogens may cause infections in insects through the external openings, through the integument, and by congenital transmission. These methods may be modified as in the case of transmission through the stings of insect parasites or bites of predators, or through cannibalism. In recent years, much attention has been placed on latent infections, especially with virus, transmitted from generation to generation under natural conditions.

Dresner (50) has been the first to report that the germinating conidia of *Beauveria bassiana* produce a contact toxin when used against certain insects. Schaerffenberg (151) has confirmed the presence of a toxin, but limited attempts by Steinhaus & Bell (171) were inconclusive. A repellent effect has been observed when the spores and crystals of *Bacillus thuringiensis* and its close relatives were tested on young larvae of several insects (5, 73, 82). However, *B. thuringiensis* spores had no repellent effect on the first-instar larva of the European cornborer [McConnell & Cutkomp (127)]. Inasmuch as repellent effects and quick-acting contact toxins would be of special value in insect control, especially on crops with a low economic threshold, further investigations along these lines with *Bacillus* spp. and *Beauveria* spp. may be highly profitable.

Since the works of Paillot, additional observations have appeared on the transmission of protozoa by insect parasites (29, 30, 112, 185, 197). Maternal transmission of protozoa through the egg has been observed in the European cabbageworm [Blunck (29)], the imported cabbageworm [Tanada (185)], the European corn borer [Zimmack *et al.* (224); Zimmack & Brindley (225)], the spruce budworm, *Choristoneura fumiferana* (Clemens) [Neilson (131); Thomson (196)], the fall webworm [Machay (114); Weiser (214)], and the alfalfa snout beetle, *Brachyrhinus ligustici* (Linnaeus) (= *Otiorhynchus ligustici* Linnaeus) [Weiser (215)]. Apparently microsporidia are not transmitted through the infected males of the European corn borer [Zimmack *et al.* (224)] and the silkworm, *Bombyx mori*

(Linnaeus) [Machay (115)], but are transmitted to a certain extent by infected males of the spruce budworm [Thomson (196)]. Weiser (212), however, considers that congenital transmission of protozoa occurs only in a few cases in wild insects. The bacterium, *Pseudomonas aeruginosa,* is intimately associated and transmitted with a small percentage of grasshopper eggs [Bucher & Stephens (35)]. In the nun moth, *Lymantria monacha* (Linnaeus), the unidentified *Schlaffsucht* bacteria are transmitted through the egg [Janisch (99)].

An increasing number of reports have appeared on the transmission of viruses through the egg. There is still some doubt whether the virus transmission within the egg has been distinctly established in the laboratory [Smith *et al.* (160)]. For a thorough discussion on virus transmission, the reader is referred to the review by Bergold (15).

One of the controversial problems in microbial control, especially in the case of virus diseases, is that of latency. The confusion exists to some extent because of insufficient knowledge of the process of latent infections in insects. Some insect pathologists consider latency as the only possible answer to certain results observed with insect viruses in the laboratory and field [see Bergold (15)]. Aside from the viruses, there are only a few reports of latency in other insect diseases. The reports of latent bacterial infections in the nun moth [Janisch (99)] and of latent microsporidian infections in the spruce budworm [Bergold (13)] and in the African migratory locust, *Locusta migratoria migratoriodes* Reiche & Fairmaire [Canning (39)], need further confirmation.

Some investigators consider latent virus infections widespread throughout the population of certain forest insects (14, 15, 65, 74, 99, 107, 109, 147, 206). Assuming that such widespread infections exist in nature, Roegner-Aust (147) has expressed the futility of the artificial application of viruses for the direct control of the latently-infected insects. It has also been implied that the successful field control of insects with viruses may have resulted from virus activation through superinfections [see Bergold (15)]. But this is highly unlikely in many cases, because the application of virus has resulted in rapid development of infection, especially among insects which have shown no indication of latent infections. It must be realized, however, that under certain conditions, the initiation of virus epizootics is probably connected with environmental stresses (103, 104, 107, 109, 110, 123, 182, 210) and the use of such stresses has been advocated for insect control. Steinhaus (170) has observed that certain stressors and incitants may increase the incidence of diseases among some insects.

Methods of Dispersal

Insect pathogens do not possess properties comparable to the searching capacities of insect parasites and predators. Possible exceptions may be the entomogenous nematodes and fungi, but it is unlikely that they exhibit a definite search pattern, and host infection or discovery occurs mainly at

random. The effectiveness of insect pathogens depends to a large extent on factors governing their dispersal capacity and pathogenicity. One of the major objectives of microbial control is to augment and facilitate the dispersal of insect pathogens.

Dispersal in nature occurs by various methods, such as by the movements of infected hosts (primary, secondary, etc.), by being transported on the bodies of insects and animals, by climatic and physical factors (wind, rain, stream, etc.), and by their own motility or by special discharging apparatus (fungi).

Dispersion by the movement of infected primary and secondary hosts is the principal method of dissemination of insect pathogens. This method has received extensive investigation with forest insect pathogens, especially viruses and protozoa (*26*, 40, *42*, 65, *67*, *212*). Insects with virus infections having relatively long periods of infection, such as the spruce budworm, are more capable of distributing the infection throughout the population [Bergold & Bird (16)]. The ovipositional characteristic of the insect species may also influence virus distribution. Bird (*26*) has observed that, at low population levels, the virus is distributed more effectively by infected European spruce sawfly, which lays its eggs singly, than by European pine sawfly, whose eggs are laid in clusters. Thus the virus of the former species is deposited in a wider area and has more foci of infections than the virus of the latter.

Recent observations on the distribution of entomophthorous fungi have indicated that the infected alates of the spotted alfalfa aphid [Hall & Dunn (80)] and the pea aphid, *Macrosiphum pisi* (Harris) [MacLeod (119); Rockwood (145)], are largely responsible for the distribution of these fungi. In the fungus, *Zygaenobia intestinalis* Weiser, the conidia are discharged into the larval gut of *Zygaena carniola* Scopoli, and the infected larva distributes the conidia through the feces [Weiser (211)]. Such a method of distribution is reminiscent of the one in *Massospora*. In a survey conducted by trapping adult calyptrate flies, several of the species have been found to carry *Empusa* spp. [Judd (100)].

As mentioned previously, certain microsporidia and bacteria are transmitted through the hosts, and the movements of these infected hosts distribute the pathogens. Majumder *et al.* (121) have obtained some spread of bacterial disease by liberating larvae infected with *B. thuringiensis* among the population of the lablab podborer, *Adisura atkinsoni*. Weiser (212, 214) has pointed out the importance of alternate hosts for the spread and circulation of microsporidia in some forest insects. As an alternate host of the nematode, *Neoaplectana leucaniae* Hoy, the adults of *Costelytra zealandica* (White) may distribute the nematode to fresh foci [Hoy (95)].

Insects parasites and predators, and other animals and birds may disperse viruses (23, 63, 66, 113, 194), microsporidia (29, 30, 112, 185, 197, 212), and bacteria (141).

Host Resistance

In general, young larvae are more susceptible than older larvae to most diseases (76, 78, 127, 182, 183, 187, 201, 209). In the European spruce and pine sawflies, the prepupal stage is immune to infection by polyhedrosis viruses [Bird (24); Bird & Whalen (28)]. The temporary prepupal mid-gut is composed of resistant embryonic cells, but the new digestive cells which appear later in the pupa and adult are susceptible to the viruses.

Older pupae of some Lepidoptera are resistant to infection by *Beauveria bassiana, Aspergillus flavus* Link, and *Aspergillus luchuensis* Inui [Sussman (179); Tanada (186)], and their resistance is apparently accounted for by a waxy epicuticular layer [Sussman (179); Koidsumi (102)] in which the free medium-chain saturated fatty acids, presumably caprylic or capric acids, seem to act as antifungal agents [Koidsumi (102)].

The susceptibility of certain insects to the entomogenous strains of *Bacillus cereus* (noncrystal-bearers) and to *B. thuringiensis, B. sotto* and their close relatives (crystal-bearers) is apparently associated with pH of the mid-gut. Insects susceptible to the noncrystal-bearers had mid-gut pH (less than pH 9) close to the optimum (pH 6.6 to 7.4) for the toxic enzyme, lecithinase, produced by these bacteria [Heimpel (92)], and those susceptible to the crystal-bearers had mid-gut pH toward the alkaline (pH 9.0 to 10.5) which is necessary to dissolve the toxic crystals [Angus (2, 5)].

The general assumption is that adult insects are resistant to acute virus infections even though they may transmit the virus in an active or latent state to their offspring. When mated with healthy males, the females of the fall webworm which have developed from larvae presumably with virus infections have laid fewer number of eggs than females from uninfected larvae [Szirmai (182)]. But, it was not determined whether the reduction in fecundity was caused by the direct effect of the virus on the adult or by the effect of the virus on the larval development in such a way as to weaken the adult. Microsporidian infections in adults are known to reduce egg production in the spruce budworm [Neilson (131); Thomson (195)] and the European corn borer [Zimmack *et al.* (224); Zimmack & Brindley (225)].

Very little information is available on the occurrence of resistant strains of wild insects or on the acquired resistance of wild insects to disease, despite the long association between the hosts and pathogens. Thus far, there is no clear-cut case of insect pests becoming resistant to disease. Bird & Elgee (27) have speculated that the recent continual increase of the European spruce sawfly without a subsequent rapid rise in polyhedrosis suggests for the first time an increase in the resistance of the sawfly to the disease. Martignoni (123) has observed that in a population of the larch bud moth the LD_{50} of a granulosis virus increased from 1954 to 1955. This seems to indicate that the larch-bud-moth population which had been

declining because of a granulosis epizootic since 1954 has apparently developed some resistance to the virus. In connection with the immunity to pathogens acquired by insects, it should be pointed out that the pathogens would also be capable of mutating to more or less virulent forms.

Continuous rearing at high temperatures seems to increase the resistance and to cause the apparent recovery of the imported cabbageworm [Tanada (183)] and the European spruce sawfly [Bird (25, 26)] from virus infections. However, these insects become susceptible to the virus when the temperature is lowered or alternated at high and low.

Virulence

There is no definitely established record of insect virus showing a loss in virulence. The viruses of the European spruce and pine sawflies have apparently maintained their virulence up to the present [Bird (26); Krieg (109)]. A possible strain difference in the polyhedrosis virus of the wattle bagworm, *Kotochalia junodi* (Heylaerts), has been observed by Ossowski (138, 139). He has noticed that virus collected from infected larvae in plantations that are distant from the area of application causes a higher kill than virus obtained from the area on which it is applied. Although this may be indicative of a strain difference, further tests should be conducted, especially with dosage-mortality analysis.

Strains with varying degrees of virulence are known for *Bacillus thuringiensis* and its close relatives (6, 111, 120, 162), for *Beauveria* spp. (117, 118), and for *Metarrhizium anisopliae* (144, 146). Sussman (180) has found that quantitative studies of virulence can be based on the respiratory gas exchange and on the weight loss in the insect pupa during infection with a fungus. This method should be investigated further, especially with other pathogens beside fungi.

Although *B. thuringiensis* isolated by Mattes in 1927 has shown no loss in virulence after repeated transfers on nutrient agar [Steinhaus (162)], certain strains and closely related forms isolated by other workers (111, 121, 199, 204, 208) have shown more or less marked loss in virulence when repeatedly grown on artificial media, but they all have regained their virulence when passed through a susceptible host. Such loss and gain in virulence have been observed also in the entomogenous strains of *Bacillus cereus* [Stephens (174, 175); Toumanoff (200)] and other sporeformers [Wikén & Willie (221)].

An increase in the number of virus bundles in a polyhedron after several passages of the virus through the fall webworm has been reported by Machay & Lovas (116) and by Szirmai (182). In this case, the virulence of the virus may not necessarily be increased, as conjectured by these workers, because virulence should be based not on the amount of polyhedra but on the amount of the virus itself.

The possibility that the virulence of certain pathogens can be in-

creased by combination with certain incitants and with other pathogenic or nonpathogenic microorganisms has been considered by several investigators [e.g., Steinhaus (170)]. In the armyworm, *Pseudaletia unipuncta* (Haworth), the susceptibility of the older larvae to virus infections has been greatly increased by feeding them a mixture of a granulosis and a polyhedrosis virus [Tanada (187)]. Isakova (97) has found a complex culture of bacteria which is more pathogenic to several species of insects than the individual bacterial species. In the case of protozoa, a mixed infection by two or more microsporidian species in the gypsy moth, *Porthetria dispar* (Linnaeus), is not more virulent than infection by the individual microsporidian [Weiser (212)]. However, some host-specific microsporidia (e.g., *Nosema lymantriae* Weiser) can be transferred to other hosts [e.g., *Euproctis chrysorrhoea* (Linnaeus)] by combining with a microsporidian (e.g., *Thelohania similis* Weiser) pathogenic for these hosts [Weiser (212, 213)]. This is the first recorded case of synergism between microsporidia in insects.

The storage capacity of the different pathogens is obviously important in microbial control. In general, insect viruses within their inclusion bodies can be stored in a dried state from several months to several years, and when held in water at 4°C., they remain viable for several years [see Bergold (15)]. The dried spores of *Bacillus thuringiensis* have remained viable for a least six years [Steinhaus (162)], and those of the entomogenous *Bacillus cereus* have retained their virulence for 1 to 2 years [Stephens (174); Heimpel (91)]. The spores of *Beauveria* gradually lose their vitality when stored in a dried state [Masera (126)] and have retained their virulence best at temperatures of 5°C. and − 30°C. [Hall (76)].

Protozoa and nematodes are more sensitive to storage conditions than bacteria and fungi. When dried, they lose their viability within several hours to about one to two months (51, 76, 214). Protozoan spores suspended in water have remained viable at room temperatures for three to four months and rarely more than 10 months, but at 0°C., they are viable for more than 13 months [Weiser (212, 214)]. The nematode, *Neoaplectana glaseri* Steiner, when cultured for seven years in axenic culture during which an estimated 180 to 195 generations had been passed *in vitro*, was still capable of infecting the Japanese beetle [Stoll (177)]. *Neoaplectana leucaniae* Hoy has retained its virulence on artificial culture for as many as 10 generations [Hoy (95)].

Methods of Application

There are four major ways to apply pathogens for insect control: (*a*) applied alone as a spray, dust, or bait; (*b*) introduction and colonization into an insect population; (*c*) application in combination with insecticides, both compatibly and synergistically; and (*d*) application combined or co-ordinated with insect parasites, predators, and other microorganisms.

Thus far, the first two methods have been used most frequently, and only a few attempts have been made in combination with insecticides, parasites, or other microorganisms.

Most of the present-day conventional sprayers and dusters, mist blowers, and aircraft have been found suitable for the application of insect pathogens, especially viruses and bacterial spores. Insect pathogens are usually applied in their resistant state or stage: the viruses within their inclusion bodies (polyhedra or capsules); bacteria, fungi, and protozoa as spores; and the nematodes in their resistant *dauer* stage. Nematodes and protozoa are apparently capable of withstanding the usual pressures of sprayers (51, 212, 214, 216). The nonsporeforming bacterium, *Pseudomonas aeruginosa,* has been used against grasshoppers in sprays and baits together with a protectant [Baird (8)].

Dosage-mortality analyses have been conducted mainly with viruses and spore-forming bacteria (7, 13, 15, 93, 106, 116, 123, 127, 184). A microinjector glass needle for the determination of LD_{50} by way of the mouth has been developed by Martignoni (122), and a settling tower comparable to the one for testing insecticides has been constructed by Burgerjon (36, 37).

With sporeforming bacteria, the dosage-mortality analyses are complicated by the actions of toxic crystal (3, 7, 86, 87, 88, 172), of toxic enzyme [Heimpel (93)], of the spore itself [Toumanoff (198)], and possibly by the combination of these methods [Toumanoff & Vago (203); Toumanoff (198)].

Several workers have also adopted the median lethal time (LT_{50}) to express the effectiveness of the insect pathogens [Krieg (106, 108, 110); Biliotti (20)]. Inasmuch as different methods, some of them certainly inappropriate, have been used in the dosage-mortality analysis of insect pathogens, it is recommended that these methods be standardized for each group of pathogens and conducted under controlled conditions.

The proper timing of applications is generally much more critical in the use of insect pathogens than in the use of insecticides because of the relatively long period from infection to the death of the host, and during this period the insect may continue to feed on the plants. Timing is generally critical in the application of viruses (23, 43, 124, 128, 192, 194), but for the control of the Great Basin tent caterpillar [Clark & Reiner (43)] and the cabbage looper [McEwen & Hervey (128)], timing of the virus application may not be a limiting factor.

Timing, however, is not as critical with the use of certain bacteria, such as *Bacillus thuringiensis, B. sotto,* and their close relatives, which cause rapid death, within one to five days, and often quickly inhibit the feeding of the insects (5, 108, 162, 188, 193, 201).

In recent years, the introduction and colonization of insect pathogens have been highly successful with the polyhedrosis viruses of certain saw-

flies [Bird (26)], with the milky-disease organisms of the Japanese beetle [Polivka (141)], and with the fungi of the spotted alfalfa aphid [Hall & Dunn (80, 83)].

Persistence in the Field

There are only limited observations on the persistence of insect pathogens in the field. Most of the related investigations on the longevity of pathogens have been conducted under laboratory conditions and have not been incorporated into this review.

Many pathogens persist in an insect population within infected hosts. Viruses of the European spruce and pine sawflies, once they are introduced artificially into a susceptible host population, spread (in one case over 500 feet in one year) and persist for many years (11, 23, 26, 27). These viruses are apparently incapable of surviving on the plants during the winter [Bird (26)]. But in the case of the Great Basin tent caterpillar, the virus may persist over winter, not only in infected individuals, but also in the cadavers of virus-killed larvae [Clark (40, 41, 42)]. Thompson & Steinhaus (194) have found that the soil in alfalfa fields contained polyhedrosis virus that is infectious for the alfalfa caterpillar. Although no tests were conducted, it is highly probable that the virus may persist in the soil for some years. Apparently the polyhedrosis virus of the wattle bagworm persists in the field for several years [Ossowski (137)]. Field observations made at regular intervals have indicated that the milky-disease organisms persist for at least nine to ten years when used against the Japanese beetle and the European chafer (141, 190, 219). These examples of relatively permanent establishment of insect pathogens in the field certainly do not agree with Ullyett's (205) contentions that diseases are only temporary and not permanent factors of control.

Stephens (175) has attempted to evaluate the coverage and persistence of *Bacillus cereus* spores when sprayed on apple trees for codling-moth control. She found that some spores apparently germinated and the bacteria multiplied after the second and later sprays, and the variation in persistence was caused mainly by factors that affected the viability of the bacteria. In field tests on the European cabbageworm, the spores of *B. cereus* var. *alesti* have persisted only for a week [Toumanoff & Grison (201)], but those of *B. thuringiensis* ("Anduze" strain) have persisted even after rains had fallen, and in one case they have prevented cabbageworm infestation for a longer period than two applications of DDT [Lemoigne *et al.* (111)].

A protectant, composed of 1 per cent casein, 5 per cent sucrose and 1 per cent granular mucin, when used with the nonsporeforming bacterium, *Pseudomonas aeruginosa,* has preserved 29 per cent of the bacteria after 24 hours and about 9 per cent after 1 week [Stephens (176)]. Such investigations for suitable protectants may eventually bring about the field application of some of the highly virulent nonsporeforming bacteria.

The white muscardine fungus, when applied to the hibernating quarters of the bug *Aradus cinnamomeus* Panzer, has remained effective for at least

two years, and the fungus infection has spread out considerably from the treated foci [Smirnov (158)]. The fungi of the spotted alfalfa aphid when introduced into a fungus-free population have persisted for several years, and even during periods of host scarcity [Hall & Dunn (80); Hall (79)].

The entomophthorous fungi would probably be applied more widely, if there were more knowledge on the production and germination of their thick-walled resting spores. Hall & Halfhill (85) have observed that some (2 to 5 per cent) of the resting spores of *Entomophthora virulenta* Hall & Dunn are ready to germinate immediately when removed from the dry state to an artificial media, and others have germinated progressively, as the period of exposure to high moisture is increased.

Some microsporidian spores and the resistant *dauer* stages of nematodes are capable of surviving in moist soil or in cadavers in the soil for one or more years (75, 95, 214).

Effect of Physical Environmental Factors

There is still much ignorance of the effects of the various physical environmental factors in microbial control. These factors may affect the pathogen (its survival and ability to infect), the host (its susceptibility or resistance, including activation of latent infections), and the progress of infection within the host. In the present review, the effect of environmental factors on the progress of infection has been considered to a limited extent in the section on resistance.

Observations are accumulating on the importance of high humidity with or without accompanying high temperature in fungus infections (48, 80, 130, 134, 135, 138, 145, 223). Some reports have pointed out the importance of humidity in the microclimate [Domenichini & Vago (48); Hall & Dunn (80)], and fungus epizootics among spotted alfalfa aphids have been initiated by the proper timing of irrigation in alfalfa fields [Hall & Dunn (80)]. By means of a specially built apparatus, Hart & MacLeod (89) have observed that optimum germination of *Beauveria bassiana* spores occurs at relative humidity above 94 per cent and at a temperature of 28°C., with negligible germination at 10°C., 38°C., and 44°C.

The white grubs of *Melolontha melolontha* living in rich organic soils have been readily infected by the application of an unidentified milky-disease organism, but those living in sandy or clay soils have not been infected to any appreciable extent [Hurpin (96)]. In the case of the European chafer, *Amphimallon majalis*, the rate of infection by milky-disease organisms is directly related to the soil temperature [Tashiro & White (190); Tashiro (189)].

According to Weiser (212), weather and host food plant have no affect on microsporidia. Nematodes, like the fungi, are greatly dependent on high humidity and moisture, especially during periods of dispersal outside of their hosts (46, 51, 154). The promising nematode isolated from codling moth would probably not be effective against insects exposed to the drying

effect of the atmosphere [Dutky (51)]. Some entomogenous nematodes (mermithids) apparently favor calcareous soils [Théodoridès (191)].

The incidence of virus infections in such hosts as the European pine and spruce sawflies (26, 27, 105) is apparently unaffected by environmental temperature and humidity. On the other hand, virus activations resulting from environmental stresses have been reported by several workers, especially in forest insects, such as the gypsy moth, the nun moth, and others (15, 65, 99, 210), but concrete evidence is generally lacking in most of these reports. Wallis (210) has recently reported a direct correlation between high humidity and the appearance of virus epizootics among gypsy-moth populations. However, Shvetsova (156), Schmidt (152), and Kovačević (104) have observed that virus activation in the gypsy moth in Europe is dependent mainly on the type of food plant and is independent of the humidity. Although in these cases the response to different stresses may result from differences in the strains of gypsy moth, further confirmation on virus activation by environmental factors would be desirable, with special attention paid to the time of virus activation rather than to the period of morbidity. The possible association between high humidity and the development of virus epizootics has also been indicated in the fall webworm [Szirmai (182); Schmidt & Philips (153)], the gamma noctuid [Vago & Cayrol (207)], and the California oakworm, *Phryganidia californica* Packard [Harville (90)].

Compatibility with Adjuvants, Insecticides, Fungicides, and Antibiotics

Certain insect pathogens, especially viruses and bacteria, have been combined satisfactorily with various adjuvants (wetting and sticking agents), such as blood albumin, milk powder, wheat flour, glycogen, and several of the recent proprietary surface-active agents (4, 23, 44, 110, 143, 179, 188, 212). These adjuvants usually increase the effectiveness of the insect pathogens.

The detrimental effect of insecticidal and fungicidal spray residues on the entomogenous fungi of scale insects and mites has been extensively investigated in Florida (60, 71, 129, 130). Wettable sulfur appears less harmful than lime-sulfur and hydrated lime to *Myiophagus* sp. and *Hirsutella besseyi* Fisher, which parasitize purple scales [Fisher & Griffiths (60)]. Acaricidal sulfur may be unfavorable for an *Entomophthora* on the citrus red mite [Muma (130)]. Systox at a high concentration of 1 per cent has prevented the development of most soil microorganisms (60 species tested *in vitro*) but at the concentrations (0.1 to 0.001 per cent) used in the field, it may even have a stimulating effect for certain soil microorganisms [Swart-Füchtbauer (181)].

The five species of fungi pathogenic for the spotted alfalfa aphid react diversely when combined with several insecticides and fungicides [Hall & Dunn (84)]. The most effective pathogen, *Entomophthora exitialis* Hall &

Dunn, seems the most susceptible, and *Entomophthora coronata* (Costantin) Kevorkian the least susceptible. Parathion and DDT appear to be the most innocuous of the five insecticides tested, and Trithion is lethal to all five fungi. The germination of resting spores of *Entomophthora virulenta* Hall & Dunn is retarded for about seven days when they are exposed to the five insecticides.

Glass (70) has found that the granulosis virus of the red-banded leaf roller is unaffected by the fungicides, sulfur, ferbam, captan, dichlone, and phenyl mercuric acetate, but glyodin has reduced the incidence of the disease. In field tests on the cabbage looper, the addition of TEPP to the polyhedrosis-virus spray suspension seems to have no adverse affect on virus activity [McEwen & Hervey (128)]. Weiser (212) has reported that insecticides have no unfavorable influence on the development of microsporidia within their hosts. The nematode isolated from codling-moth larva is resistant to the action of several insecticides and fungicides [Dutky (51)].

Inasmuch as antibiotics are used against certain plant diseases, it is of interest that insect viruses and microsporidian spores are generally unaffected by the common antibiotics, such as penicillin, chlortetracycline (aureomycin), streptomycin, and oxytetracycline (terramycin) [Weiser (212); Bergold (15)]. *Bacillus thuringiensis, B. sotto,* and their close relatives, however, show marked variations in their reactions to the antibiotics [Toumanoff & Lapied (202)]. Penicillin seems innocuous, but streptomycin affects most of them.

One of the promising areas of microbial control is the use of disease to increase the susceptibility of the insects to insecticides. Increased susceptibility to contact and stomach insecticides has been observed in microsporidian-infected beetles of *Brachyrhinus ligustici* [Rosický (148); Weiser (212)].

Compatibility with Other Biological Control Agents

Insect parasites and predators are generally unaffected by diseases of their hosts, and they occur commonly in areas where epizootics are reducing their insect hosts. The regulation of insect populations by the combined effect of diseases and parasites is evident in such outstanding examples as in the control of the alfalfa caterpillar [Thompson & Steinhaus (194)], the sawflies [Bird & Elgee (27); Balch (10)], and the spotted alfalfa aphid [Hall & Dunn (80)]. Occasionally the virus may control the host population at such a low level that certain parasites have been eliminated from the area. But in some of these cases (e.g., the European spruce sawfly), other parasites more capable of maintaining themselves at low host densities have taken their places [Bird & Elgee (27)]. Some parasites move out to the periphery of an epizootic area where more hosts are available, whereas others are able to complete their development in diseased host larvae and survive the epizootic [Franz & Krieg (65)]. Thompson & Steinhaus (194) have

emphasized the importance of the timing of virus and bacterial sprays in conserving the insect parasites and bringing about more effective control of the alfalfa caterpillar.

It is generally assumed that insect pathogens usually do not directly attack the insect parasites and predators of their hosts. Several insect parasites and predators have been observed developing on hosts infected with bacteria and a virus [Biliotti (17, 18, 19); Tanada (188)]. Spores of *Bacillus thuringiensis* do not affect the adults and colonies of *Polistes exclamans* Viereck, an important natural enemy of hornworms [Rabb (142)]. As mentioned previously, certain predators and birds are unaffected by feeding on insects with virus and protozoan diseases.

Direct infection of insect parasites has been observed with the microsporidia of the cabbageworms [Blunck (29, 30); Tanada (185)], of the fall webworm [Weiser (214)], of the potato tuberworm [Allen (1)], and of *Aporia crataegi* (Linnaeus) [Lipa (112)]. In the case of the potato tuberworm, *Nosema destructor* Steinhaus & Hughes may seriously affect the insectary production of the beneficial parasite, *Macrocentrus ancylivorus* Rohwer [Allen (1)]. Inasmuch as the predators of the fall webworm select microsporidian-infected larvae over healthy ones, their actions may be harmful because they remove the potential source of infectious material from the area [Weiser (212); Weiser & Veber (217)]. On the other hand, Masera (125) has observed that *Apanteles glomeratus* (Linnaeus) is apparently capable of selecting healthy European cabbageworms from those infected with pébrine (microsporidian infection).

Mixed or multiple infections of several different pathogens within the same host are frequently observed under laboratory conditions. The mixed infections may vary from coexistence to antagonism or to synergism. Lists of examples of two or more viruses occupying the same host insect have been compiled by Martignoni (123) and Steinhaus (167).

One of the areas of research in which there will undoubtedly be more field tests in the future is the use of two or more different pathogens in the control of one or more insect hosts. For the control of the alfalfa caterpillar, a mixture of virus and bacillus spores has caused a faster reduction in population than the virus alone, but the control obtained has not been as complete or long lasting as that from the use of only the virus [Thompson (193)]. In the control of the *Melolontha* sp., no observable additive effect has been detected when a bacterium and a fungus, both of them pathogens of *Melolontha*, were used together [Wikén *et al.* (220)]. Although these two field tests have indicated no increase in effectiveness by the combination of pathogens, they should not discourage further attempts along these lines.

Insect Disease as a Population Mortality Factor

When used as "living or microbial insecticides," insect pathogens, like chemical insecticides, may act as density-independent mortality factors. But, unlike chemical insecticides, highly virulent pathogens when applied to a

small portion of a susceptible host population can multiply and spread as a density-dependent mortality factor [Bird (23, 25); Hall & Dunn (80)].

It is generally accepted that, under natural conditions, the disease-producing microorganisms affect a greater proportion of the insects as the host population increases in density, i.e., they are density-dependent mortality factors. However, in recent years, the investigations on the viruses of the sawflies [Bird & Elgee (27)] and of the Great Basin tent caterpillar [Clark & Thompson (45); Clark (40)] and on the fungi of the spotted alfalfa aphid [Hall & Dunn (80)] have indicated that epizootics can occur at low host densities. Apparently, after a severe epizootic in an extensive population of susceptible hosts over a wide area, these pathogens are capable of preventing the increase of the following host generation which may be at a low density.

Epizootics may also depend, not directly on the absolute host density, but on the relative density determined in relation to the environmental capacity for the species [Martignoni (123)]. The population of the larch bud moth, when approaching the capacity of the biotope, is apparently affected by stress factors, such as starvation, which encourage the development of virus epizootics [Martignoni (123)]. Among insects with latent virus infections, certain environmental factors may possibly trigger epizootics independent of the host density (99, 104, 147, 210). However, the effect of such factors needs further substantiation. From these examples, "It can be assumed that density-dependent and density-independent mortality factors are both involved in the totality of epizootic dynamics but there is little reason to question the generalization that disease is essentially a density-dependent mortality factor" [Steinhaus (163)].

LITERATURE CITED

1. Allen, H. W., *Ann. Entomol. Soc. Am.*, **47,** 407–24 (1954)
2. Angus, T. A., *Can. Dept. Agr. Forest Biol. Div. Sci. Ser., Bi-Monthly Progr. Rept.*, **10**(4), 2–3 (1954)
3. Angus, T. A., *Nature*, **173,** 545–46 (1954)
4. Angus, T. A., *Can. Entomologist*, **86,** 206 (1954)
5. Angus, T. A., *Can. Entomologist*, **88,** 280–83 (1956)
6. Angus, T. A., *Can. J. Microbiol.*, **2,** 111–21 (1956)
7. Angus, T. A., *Can. J. Microbiol.*, **2,** 122–31 (1956)
8. Baird, R. B., *Can. Entomologist*, **90,** 89–91 (1958)
9. Baird, R. B., *Proc. Intern. Congr. Entomol., 10th Meeting* (Montreal, Que., Canada, 1956), **4,** 689–92 (1958)

9a. Baird, R. B., *The Artificial Control of Insects by Means of Entomogenous Fungi: A Compilation of References with Abstracts* (Entomology Laboratory, Belleville, Ont., Canada, mimeo., 53 pp., 1958)

10. Balch, R. E., *Ann. Rev. Entomol.*, **3,** 449–68 (1958)
11. Benjamin, D. M., Larson, J. D., and Drooz, A. T., *J. Forestry*, **53,** 359–62 (1955)
12. Bergold, G. H., *Can. Dept. Agr. Forest Biol. Div. Sci. Ser., Bi-Monthly Progr. Rept.*, **7**(4), 1–2 (1951)
13. Bergold, G. H., *Can. J. Zool.*, **29,** 17-23 (1951)
14. Bergold, G. H., *Advances in Virus Research*, **1,** 91–139 (1953)
15. Bergold, G. H., *Handb. Virusforsch.*, **4,** 60–142 (1958)
16. Bergold, G. H., and Bird, F. T., *Can. Dept. Agr. Forest Biol. Div. Sci. Ser., Bi-Monthly Progr. Rept.*, **5**(3), 2–3 (1949)
17. Biliotti, E., *Compt. rend.*, **240,** 1021–23 (1955)
18. Biliotti, E., *Entomophaga*, **1,** 45–53 (1956)
19. Biliotti, E., *Entomophaga*, **1,** 101–3 (1956)
20. Biliotti, E., *Entomophaga*, **1,** 95–98 (1956)
21. Biliotti, E., Grison, P., and Martouret, D., *Entomophaga*, **1,** 35–44 (1956)
22. Biliotti, E., Grison, P., and Vago, C., *Compt. rend.*, **243,** 206–8 (1956)
23. Bird, F. T., *Can. Entomologist*, **85,** 437–46 (1953)
24. Bird, F. T., *Can. J. Zool.*, **31,** 300–3 (1953)
25. Bird, F. T., *Can. Dept. Agr. Forest Biol. Div. Sci. Ser., Bi-Monthly Progr. Rept.*, **10**(1), 2–3 (1954)
26. Bird, F. T., *Can. Entomologist*, **87,** 124–27 (1955)
27. Bird, F. T., and Elgee, D. E., *Can. Entomologist*, **89,** 371–78 (1957)
28. Bird, F. T., and Whalen, M. M., *Can. Entomologist*, **85,** 433–37 (1953)
29. Blunck, H., *Symposia Intern. Congr. Entomol., 9th Meeting*, **1,** 432–38 (1952)
30. Blunck, H., *Z. angew. Entomol.*, **36,** 316–33 (1954)
31. Blunck, H., *Proc. Intern. Congr. Entomol., 10th Meeting* (Montreal, Que., Canada, 1956), **4,** 703–10 (1958)
32. Bovey, P., *Proc. Intern. Congr. Entomol., 10th Meeting* (Montreal, Que., Canada, 1956), **4,** 123–31 (1958)
33. Breny, R., *Parasitica (Gembloux)*, **7,** 118–24 (1951)
34. Bucher, G. E., *Proc. Intern. Congr. Entomol., 10th Meeting* (Montreal, Que., Canada, 1956), **4,** 695-701 (1958)
35. Bucher, G. E., and Stephens, J. M., *Can. J. Microbiol.*, **3,** 611-25 (1957)

36. Burgerjon, A., *Ann. inst. natl. recherche agron.* [C]**7,** 675–84 (1956)
37. Burgerjon, A., *Entomophaga,* **2,** 129–35 (1957)
38. Cameron, J. W. M., *Ann. Rept. Entomol. Soc. Ontario, 82nd,* 7–14 (1951)
39. Canning, E. U., *Parasitology,* **43,** 287–90 (1953)
40. Clark, E. C., *Ecology,* **36,** 373–76 (1955)
41. Clark, E. C., *Ecology,* **37,** 728–32 (1956)
42. Clark, E. C., *Ecology,* **39,** 132–39 (1958)
43. Clark, E. C., and Reiner, C. E., *J. Econ. Entomol.,* **49,** 653–59 (1956)
44. Clark, E. C., and Reiner, C. E., *J. Econ. Entomol.,* **49,** 703–4 (1956)
45. Clark, E. C., and Thompson, C. G., *J. Econ. Entomol.,* **47,** 268–72 (1954)
46. Couturier, A., *Ann. inst. natl. recherche agron.* [C]**1,** 13–37 (1950)
47. De, R. K., and Konar, G., *J. Econ. Entomol.,* **48,** 773–74 (1955)
48. Domenichini, G., and Vago, C., *Boll. zool. agrar. e bachicolt., Univ. studi Milano,* **21,** 83–86 (1955)
49. Dowden, P. B., and Girth, H. B., *J. Econ. Entomol.,* **46,** 525–26 (1953)
50. Dresner, E., *J. N. Y. Entomol. Soc.,* **58,** 269–78 (1950)
51. Dutky, S. R., *Agr. Research (U.S.),* **4,** 3–4 (1956)
52. Dutky, S. R., and Gooden, E. L., *J. Bacteriol.,* **63,** 743–50 (1952)
53. Dutky, S. R., and Hough, W. S., *Proc. Entomol. Soc. Wash.,* **57,** 244 (1955)
54. Evlakhova, A. A., *Akad. Sel'skokhoz. Nauk im. V. I. Lenina Inst. Agropochvovedeniya S. S. S. R.,* **18,** No. 3, 36–39 (1953)
55. Evlakhova, A. A., *Infections and Protozoan Diseases of Insects,* Abstr. of Repts., Joint Plenary Session of the Sericulture and Apiculture, Plant Protection, and Veterinary Science Sections (Poltev, V. I., Alexandrova, L. V., and Evlakhova, A. A., Eds., Vesoiuznaia akademiia sel'skokhoziaĭstvennykh nauk imeni V. I. Lenina, Leningrad, U.S.S.R., 76 pp., 1954)
56. Faldini, J. D., and Pastrana, J. A., *Rev. arg. agron.,* **19,** 154–65 (1952)
57. Fisher, F. E., *J. Econ. Entomol.,* **43,** 305–9 (1950)
58. Fisher, F. E., *Mycologia,* **42,** 290–97 (1950)
59. Fisher, F. E., *Florida Entomologist,* **34,** 83–88 (1951)
60. Fisher, F. E., and Griffiths, J. T., Jr., *J. Econ. Entomol.,* **43,** 712–18 (1950)
61. Fisher, F. E., Thompson, W. L., and Griffiths, J. T., Jr., *Florida Entomologist,* **32,** 2–11 (1949)
62. Fox, C. J. S., and Jaques, R. P., *Can. Entomolgist,* **90,** 314–15 (1958)
63. Franz, J., *Entomophaga,* **1,** 103–4 (1956)
64. Franz, J., *Entomophaga,* **1,** 104–5 (1956)
65. Franz, J., and Krieg, A., *Z. Pflanzenkrankh. u. Pflanzenschutz,* **64,** 1–9 (1957)
66. Franz, J., Krieg, A., and Langenbuch, R., *Z. Pflanzenkrankh. u. Pflanzenschutz,* **62,** 721–26 (1955)
67. Franz, J., and Niklas, O. F., *Nachrbl. deut. Pflanzenschutzdienstes (Stuttgart),* **6,** 131–34 (1954)
68. Gerhardt, R. W., *Proc. Papers Ann. Conf. Calif. Mosquito Control Assoc., 22nd Conf.,* 50–53 (Univ. of Calif., Berkeley, Calif., 1953)
69. Gerhardt, R. W., *Proc. Papers Ann. Conf. Calif. Mosquito Control Assoc., 23rd Conf., 11th Meeting,* 120–23 (Univ. of Calif., Los Angeles, Calif., 1955)
70. Glass, E. H., *J. Econ. Entomol.,* **51,** 454–57 (1958)
71. Griffiths, J. T., Jr., and Fisher, F. E., *J. Econ. Entomol.,* **43,** 298–305 (1950)
72. Grison, P., *Ann. inst. natl. recherche agron.* [C]**7,** 543–62 (1956)

73. Grison, P., and Béguin, S., *Compt. rend. acad. agr. France,* **40,** 413–16 (1954)
74. Grison, P., and Vago, C., *Compt. rend. acad. agr. France,* **39,** 485–87 (1953)
75. Günther, S., *Z. angew. Zool.,* **43,** 397–405 (1956)
76. Hall, I. M., *Hilgardia,* **22,** 535–65 (1954)
77. Hall, I. M., *J. Econ. Entomol.,* **48,** 675-77 (1955)
78. Hall, I. M., *J. Econ. Entomol.,* **50,** 551–53 (1957)
79. Hall, I. M. (Personal communication, 1958)
80. Hall, I. M., and Dunn, P. H., *Calif. Agr.,* **11,** 5, 14 (1957)
81. Hall, I. M., and Dunn, P. H., *Hilgardia,* **27,** 159–81 (1957)
82. Hall, I. M., and Dunn, P. H., *J. Econ. Entomol.,* **51,** 296–98 (1958)
83. Hall, I. M., and Dunn, P. H., *J. Econ. Entomol.,* **51,** 341–44 (1958)
84. Hall, I. M., and Dunn, P. H., *J. Econ. Entomol.* (In press)
85. Hall, I. M., and Halfhill, J. C., *J Econ. Entomol.* (In press)
86. Hannay, C. L., *Nature,* **172,** 1004 (1953)
87. Hannay, C. L., *Symposium Soc. Gen. Microbiol.,* **6,** 318–40 (1956)
88. Hannay, C. L., and Fitz-James, P., *Can. J. Microbiol.,* **1,** 694–710 (1955)
89. Hart, M. P., and MacLeod, D.M., *Can. J. Botany,* **33,** 289–92 (1955)
90. Harville, J. P., *Microentomology,* **20,** 83–166 (1955)
91. Heimpel, A. M., *Can. Entomologist,* **86,** 73–77 (1954)
92. Heimpel, A. M., *Can. J. Zool.,* **33,** 99–106 (1955)
93. Heimpel, A. M., *Can. J. Zool.,* **33,** 311–26 (1955)
94. Heimpel, A. M., and Angus, T. A., *Can. J. Microbiol.,* **4,** 531–41 (1958)
95. Hoy, J. M., *Parasitology,* **44,** 392–99 (1954)
96. Hurpin, B., *Compt. rend. soc. biol.,* **149,** 1966–67 (1955)
97. Isakova, N. P., *Infections and Protozoan Diseases of Insects,* Abstr. of Repts., Joint Plenary Session of the Sericulture and Apiculture, Plant Protection, and Veterinary Science Sections (Poltev, V. I., Alexandrova, L. V., and Evlakhova, A. A., Eds., Vesoiuznaia akademiia sel'skokhoziaĭstvennykh nauk imeni V. I. Lenina. Leningrad, U.S.S.R., 76 pp., 1954)
98. Jacobs, S. E., *Proc. Soc. Appl. Bacteriol.,* **13,** 83–91 (1950)
99. Janisch, E., *Z. angew. Entomol.,* **42,** 292–306 (1958)
100. Judd, W. W., *Can. Entomologist,* **87,** 366–69 (1955)
101. Kelsey, J. M., *New Zealand J. Sci. Technol.,* [A]**38,** 644–46 (1957)
102. Koidsumi, K., *J. Insect Physiol.,* **1,** 40–51 (1957)
103. Kovačević, Ž., *Zashtita Bilja,* **23,** 3–20 (1954)
104. Kovačević, Ž., *Anz. Schädlingskunde,* **29,** 97–101 (1956)
105. Krieg, A., *Mitt. biol. Bundesanstalt Land- u Forstwirtsch. Berlin-Dahlem,* **30,** 92–95 (1955)
106. Krieg, A., *Arch. ges. Virusforsch.,* **6,** 163–74 (1955)
107. Krieg, A., *Arch. ges. Virusforsch.,* **6,** 472–81 (1956)
108. Krieg, A., *Z. Pflanzenkrankh. u. Pflanzenschutz,* **64,** 321–27 (1957)
109. Krieg, A., *Arch. ges. Virusforsch.,* **7,** 212–19 (1957)
110. Krieg, A., *Z. Pflanzenkrankh. u. Pflanzenschutz,* **64,** 657–62 (1957)
111. Lemoigne, M., Bonnefoi, A., Béguin, S., Grison, P., Martouret, D., Schenk, A., and Vago, C., *Entomophaga,* **1,** 19–34 (1956)
112. Lipa, J. J., *Acta Parasitol. Polonica,* **5,** 559–84 (1957)
113. Lower, H. F., *Australian J. Biol. Sci.,* **7,** 161–67 (1954)

114. Machay, M. L., *Rovartani Közlemények*, **7**, 155–62 (1954)
115. Machay, M. L., *Acta Vet. Acad. Sci. Hung.*, **7**, 11–18 (1957)
116. Machay, M. L., and Lovas, B., *Biol. Közlemények*, **5**, 7–18 (1957)
117. MacLeod, D. M., *Ann. N.Y. Acad. Sci.*, **60**, 58–70 (1954)
118. MacLeod, D. M., *Can. J. Botany*, **32**, 818–90 (1954)
119. MacLeod, D. M., *Can. Entomologist*, **87**, 503–5 (1955)
120. MacLeod, D. M., and Heimpel, A. M., *Can. Entomologist*, **87**, 128–31 (1955)
121. Majumder, S. K., Muthu, M., and Pingale, S. V., *Indian J. Entomol.*, **18**, 397–407 (1956)
122. Martignoni, M. E., *Science*, **122**, 764 (1955)
123. Martignoni, M. E., *Mitt. Schweiz. Anstalt Forst. Versuchswesen*, **32**, 371–418 (1957)
124. Martignoni, M. E., and Auer, C., *Mitt. Schweiz. Anstalt Forst. Versuchswesen*, **33**, 73–93 (1957)
125. Masera, E., *Actes congr. sericult. intern., 7th Meeting*, 551–55 (Alès, France) (1948)
126. Masera, E., *Agr. ital.*, **53**, (8 n. s.), 8–20 (January, 1953)
127. McConnell, E., and Cutkomp, L. K., *J. Econ. Entomol.*, **47**, 1074–82 (1954)
128. McEwen, F. L., and Hervey, G. E. R., *J. Econ. Entomol.*, **51**, 626–31 (1958)
129. Muma, M. H., *J. Econ. Entomol.*, **48**, 432–38 (1955)
130. Muma, M. H., *Proc. Intern. Congr. Entomol., 10th Meeting* (Montreal, Que., Canada, 1956), **4**, 633–47 (1958)
131. Neilson, M. M., *Can. Dept. Agr. Forest Biol. Div. Sci. Ser., Bi-Monthly Progr. Rept.*, **12**(6), 1–2 (1956)
132. Niklas, O. F., *Z. Pflanzenkrankh. u. Pflanzenschutz*, **63**, 81–95 (1956)
133. Niklas, O. F., *Z. angew. Zool.*, **45**, 103–16 (1958)
134. Nirula, K. K., *J. Econ. Entomol.*, **50**, 767–70 (1957)
135. Nirula, K. K., Radha, K., and Menon, K. P. V., *Indian Coconut J.*, **9**, 3–10 (1955)
136. Oka, I. N., *Tehn. Pertanian*, **6**, 113–34 (1957)
137. Ossowski, L. L. J., *Ann. Appl. Biol.*, **45**, 81–89 (1957)
138. Ossowski, L. L. J., *Z. angew. Entomol.*, **41**, 139–52 (1957)
139. Ossowski, L. L. J., *Nature*, **181**, 648 (1958)
140. Phillips, C. M., Bucher, G. E., and Stephens, J. M., *Can. Entomologist*, **85**, 8 (1953)
141. Polivka, J. B., *J. Econ. Entomol.*, **49**, 4–6 (1956)
142. Rabb, R. L., *N. Carolina State Coll., Agr. Exp. Sta., Inform. Note No. 118*, mimeo. rept., 11 pp. (1957)
143. Rabb, R. L., Steinhaus, E. A., and Guthrie, F. E., *J. Econ. Entomol.*, **50**, 259–62 (1957)
144. Radha, K., Nirula, K. K., and Menon, K. P. V., *Indian Coconut J.*, **9**, 83–89 (1956)
145. Rockwood, L. P., *J. Econ. Entomol.*, **43**, 704–7 (1950)
146. Rockwood, L. P., *Ann. Entomol. Soc. Am.*, **43**, 495–98 (1950)
147. Roegner-Aust, S., *Z. angew. Entomol.*, **31**, 1–37 (1949)
148. Rosický, B., *Věstník Československ. Zool. Společnosti*, **15**, 219–34 (1951)
149. Schaerffenberg, B., *Anz. Schädlingskunde*, **25**, 166–70 (1952)

150. Schaerffenberg, B., *Anz. Schädlingskunde*, **30**, 69–74 (1957)
151. Schaerffenberg, B., *Z. angew. Entomol.*, **41**, 395–402 (1957)
152. Schmidt, L., *Radnja primljena na štampanje*, **4**(12), 105–66 (1952)
153. Schmidt, L., and Philips, G., *Poljoprivredno-Šumarski Fakutet, zavod za entomologiju, Zagreb.*, No. 1, mimeo. rept., 27 pp. (1958)
154. Schvester, D., *Ann. inst. natl. recherche agron.* [C], *Numéro hors sér 8*, 162 pp. (1957)
155. Shands, W. A., Thompson, C. G., Simpson, G. W., and Wave, H. E., *J. Econ. Entomol.*, **51**, 184–86 (1958)
156. Shvetsova, O. I., *Mikrobiologiya*, **19**, 532–42 (1950)
157. Shvetsova, O. I., and Evlakhova, A. A., *Infections and Protozoan Diseases of Insects*, Abstr. of Repts., Joint Plenary Session of the Sericulture and Apiculture, Plant Protection, and Veterinary Science Sections (Poltev, V. I, Alexandrova, L. V., and Evlakhova, A. A., Eds., Vesoiuznaia akademiia sel'skokhoziaĭstvennykh nauk imeni V. I. Lenina. Leningrad, U.S.S.R., 76 pp., 1954)
158. Smirnov, B. A., *Infections and Protozoan Diseases of Insects*, Abstr. of Repts., Joint Plenary Session of the Sericulture and Apiculture, Plant Protection, and Veterinary Science Sections (Poltev, V. I., Alexandrova, L. V., and Evlakhova, A. A., Eds., Vesoiuznaia akademiia sel'skokhoziaĭstvennykh nauk imeni V. I. Lenina. Leningrad, U.S.S.R. 76 pp., 1954)
159. Smith, K. M., and Rivers, C. F., *Parasitology*, **46**, 235–42 (1956)
160. Smith, O. J., Hughes, K. M., Dunn, P. H., and Hall, I. M., *Can. Entomologist*, **88**, 507–15 (1956)
161. Steinhaus, E. A., *Principles of Insect Pathology* (McGraw-Hill Book Co., Inc., New York, N.Y., 757 pp., 1949)
162. Steinhaus, E. A., *Hilgardia*, **20**, 359–81 (1951)
163. Steinhaus, E. A., *Hilgardia*, **23**, 197–261 (1954)
164. Steinhaus, E. A., *Agr. Food Chem.*, **4**, 676–80 (1956)
165. Steinhaus, E. A., *Hilgardia*, **26**, 107–60 (1956)
166. Steinhaus, E. A., *Ann. Rev. Microbiol.*, **11**, 165–82 (1957)
167. Steinhaus, E. A., *Hilgardia*, **26**, 417–30 (1957)
168. Steinhaus, E. A., *Univ. Calif. (Berkeley) Lab. Insect Pathol.*, Mimeo. Ser. No. 4, 24 pp. (1957)
169. Steinhaus, E. A., *J. Econ. Entomol.*, **50**, 715–20 (1957)
170. Steinhaus, E. A., *Proc. Intern. Congr. Entomol. 10th Meeting* (Montreal, Que., Canada, 1956), **4**, 725–30 (1958)
171. Steinhaus, E. A., and Bell, C. R., *J. Econ. Entomol.*, **46**, 582–98 (1953)
172. Steinhaus, E. A., and Jerrel, E. A., *Hilgardia*, **23**, 1–23 (1954)
173. Steinhaus, E. A., and Thompson, C. G., *J. Econ. Entomol.*, **42**, 301–5 (1949)
174. Stephens, J. M., *Can. J. Zool.*, **30**, 30–40 (1952)
175. Stephens, J. M., *Can. Entomologist*, **89**, 94–96 (1957)
176. Stephens, J. M., *Can. J. Microbiol.*, **3**, 995–1000 (1957)
177. Stoll, N. R., *Thapar Commemoration Vol., 1953*, 259–68 (1953)
178. Sulzdalskaya, M. V., *Infections and Protozoan Diseases of Insects*, Abstr. of Repts., Joint Plenary Session of the Sericulture and Apiculture, Plant Pro-

tection, and Veterinary Science Sections (Poltev, V. I., Alexandrova, L. V., and Evlakhova, A. A., Eds., Vesoiuznaia akademiia sel'skokhoziaĭstvennykh nauk imeni V. I. Lenina. Leningrad, U.S.S.R., 76 pp., 1954)
179. Sussman, A. S., *Mycologia*, **43**, 338–50 (1951)
180. Sussman, A. S., *Mycologia*, **44**, 493–505 (1952)
181. Swart-Füchtbauer, H., *Höfchen-Briefe* (English Ed.), **8**, 28–34 (1955)
182. Szirmai, J., *Acta Microbiol. Acad. Sci. Hung.*, **4**, 31–42 (1957)
183. Tanada, Y., *Proc. Hawaiian Entomol. Soc.*, **15**, 235–60 (1953)
184. Tanada, Y., *Proc. Hawaiian Entomol. Soc.*, **15**, 159–66 (1953)
185. Tanada, Y., *Proc. Hawaiian Entomol. Soc.*, **15**, 609–16 (1955)
186. Tanada, Y., *Proc. Hawaiian Entomol. Soc.*, **15**, 617–22 (1955)
187. Tanada, Y., *J. Econ. Entomol.*, **49**, 52–57 (1956)
188. Tanada, Y., *J. Econ. Entomol.*, **49**, 320–29 (1956)
189. Tashiro, H., *J. Econ. Entomol.*, **50**, 350–52 (1957)
190. Tashiro, H., and White, R. T., *J. Econ. Entomol.*, **47**, 1087–92 (1954)
191. Théodoridès, J., *Vie et milieu*, **3**, 288–91 (1952)
192. Thompson, C. G., *J. Econ. Entomol.*, **44**, 255–56 (1951)
193. Thompson, C. G., *Proc. Intern. Congr. Entomol., 10th Meeting* (Montreal, Que, Canada, 1956), **4**, 693 (1958)
194. Thompson, C. G., and Steinhaus, E. A., *Hilgardia*, **19**, 412–45 (1950)
195. Thompson, H. M., *Can. Dept. Agr. Forest Biol. Div. Sci. Ser., Bi-Monthly Prog. Rept.*, **13**(3), 3 (1957)
196. Thomson, H. M., *Can. J. Zool.*, **36**, 309–16 (1958)
197. Toumanoff, C., *Rev. franç. apiculture*, **2**, 251–54 (1950)
198. Toumanoff, C., *Ann. inst. Pasteur*, **86**, 570–79 (1954)
199. Toumanoff, C., *Ann. inst. Pasteur*, **89**, 644–53 (1955)
200. Toumanoff, C., *Ann. inst. Pasteur*, **90**, 660–65 (1956)
201. Toumanoff, C., and Grison, P., *Acad. agr. France, Extrait du procèsverbal de la séance du 7 Avril*, 4 pp. (1954)
202. Toumanoff, C., and Lapied, M., *Ann inst. Pasteur*, **87**, 370–74 (1954)
203. Toumanoff, C., and Vago, C., *Ann. inst. Pasteur*, **83**, 421–22 (1952)
204. Toumanoff, C., and Vago, C., *Compt. rend.*, **235**, 1715–17 (1952)
205. Ullyett, G. C., *Entomol. Soc. S. Africa Mem.*, **2**, 89 pp. (1953)
206. Vago, C., *Ann. inst. natl. recherche agron.*, [C]**4**, 319–32 (1953)
207. Vago, C., and Cayrol, R., *Ann. inst. natl. recherche agron.*, [C]**6**, 421–32 (1955)
208. Vănková, J., *Folia Biol. (Prague)*, **3**, 175–82 (1957)
209. Vasiljević, L. A., *Inst. Zaštitu Bilja, Posebna Izdanja*, No. 7, 77 pp. (1957)
210. Wallis, R. C., *J. Econ. Entomol.*, **50**, 580–83 (1957)
211. Weiser, J., *Entomol. Listy (Folia Entomol.)*, **14**, 130–35 (1951)
212. Weiser, J., *Z. Pflanzenkrankh. u. Pflanzenschutz*, **63**, 625–38 (1956)
213. Weiser, J., *Z. angew. Entomol.*, **40**, 509–21 (1957)
214. Weiser, J., *Československ. Parasitol.*, **4**, 359–67 (1957)
215. Weiser, J., *Věstník Československ. Zool. Společnosti*, **22**, 10–12 (1958)
216. Weiser, J., and Veber, J., *Československ. Parasitol.*, **2**, 191–99 (1955)
217. Weiser, J., and Veber, J., *Z. angew. Entomol.*, **40**, 55–70 (1957)
218. Welch, H. E., *Proc. Intern. Congr. Entomol., 10th Meeting* (Montreal, Que., Canada, 1956), **4**, 863–68 (1958)

219. White, R. T., and McCabe, P. J., *U. S. Dept. Agr. Bur. Entomol. Plant Quarantine E. T., E-801,* 3 pp. (1950)
220. Wikén, T., Bovey, P., Wille, H., and Wildbolz, T., *Z. angew. Entomol.,* **36,** 1–19 (1954)
221. Wikén, T., and Wille, H., *Zentr. Bakteriol. Parasitenk.,* [II]**107,** 259–71 (1953)
222. Wille, H., and Martignoni, M. E., *Schweiz. Z. allgem. Pathol. u. Bakteriol.,* **15,** 470–74 (1952)
223. Wolcott, G. N., *Puerto Rico, Univ. Agr. Exp. Sta. Bull. No. 130,* 19 pp. (1955)
224. Zimmack, H. L., Arbuthnot, K. D., and Brindley, T. A., *J. Econ. Entomol.,* **47,** 641–45 (1954)
225. Zimmack, H. L., and Brindley, T. A., *J. Econ. Entomol.,* **50,** 637–40 (1957)

ON THE MODE OF ACTION OF INSECTICIDES[1]

By F. P. W. Winteringham and S. E. Lewis
Biochemistry Section, D.S.I.R. Pest Infestation Laboratory, Slough, Buckinghamshire, England

This article is a brief statment of views on the mode of action of certain insecticides in the light of information available to the authors in the spring of 1958. Recent reviews (25, 35, 52, 64, 84, 94, 100) provide *in toto* a comprehensive survey of the relevant literature up to the summer of 1956.

We suggest that in the study of insecticidal action too much emphasis has been placed in the search for a single mechanism. Some insecticides probably act in several ways at once; and it may be the particular combination of biochemical and physiological consequences of such multiple action, rather than some unique single lesion, which leads to the ultimate death of the insect. For the purpose of this argument insecticides will be grouped as follows:

(*a*). DDT and structurally related insecticides
(*b*). γ-BHC and the aldrin group
(*c*). Organophosphorus insecticides
(*d*). Miscellaneous insecticides

Groups *a*, *b*, and *c* contain insecticides to which some insect strains acquire high levels of resistance, not only to the insecticide to which they have been exposed but also to other members of the same group (14, 63). In all cases the discussion will be confined to toxic mechanisms per se, and the enzymic metabolism of insecticides resulting in toxication or detoxication will not be considered.

DDT and Related Compounds

Effects on oxidative metabolism.—There is evidence to show that DDT directly affects oxidative metabolism and that the effects *in vivo* may not be interpreted as the indirect consequences of the neuromuscular disturbances.

Johnston (49) found that, when added in alcohol, DDT, DDE, and methoxychlor at concentrations of $10^{-5}M$ inhibited the succinic oxidase system prepared from rat heart, whereas DDA had little effect. Johnston, however, found little inhibition when DDT was added in oil, and suggested that the use of oil by Judah (50) explained the negative result of the latter. In later experiments (51) Judah & Williams-Ashman found that DDT affected

[1] The following abbreviations are used in this chapter: ACH for acetylcholine; BHC for 1,2,3,4,5,6-hexachloro-cyclohexane; DDA for 2,2-*bis*(*p*-chlorophenyl)-acetic acid; DDD for 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethane; DDE for 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethylene; DDT for 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethane; DDVP for *O*,*O*-dimethyl-*O*-2,2-dichlorovinylphosphate; DFP for diisopropyl phosphorofluoridate; PAM for pyridine-2-aldoxime methiodide; TEPP for tetraethyl pyrophosphate.

neither respiration nor phosphorylation in a cyclophorase preparation from rabbit kidney, but the method of adding DDT in these experiments is not clear. Brown and his colleagues (66, 13) studied the effects of a wide range of insecticides on the cytochrome oxidase activity of *Periplaneta americana* (Linnaeus), *in vitro* and *in vivo*. No effects were demonstrated *in vivo* when the DDT in oil was injected into the live insect, and it was concluded that the slight inhibitions found *in vitro* were nonspecific artifacts. Anderson and her colleagues (2) found that DDT and the noninsecticidal DDE, added in acetone or alcohol, inhibited succinate oxidation by thoracic suspensions from *Musca domestica* Linnaeus. They concluded that this inhibition was not "a primary factor in the mode of action of DDT."

Ludwig, Barsa & Cali (62) found that cytochrome oxidase activity in homogenates of the mealworm, *Tenebrio molitor* Linnaeus, and of the leg of the cockroach, *P. americana*, was inhibited by $10^{-3}M$ DDT, and that the inhibition was increased by incubating the homogenates with DDT. Succinate oxidation was inhibited only when dilute homogenates were used, and Ludwig *et al.* suggested that this might result from the high cytochrome oxidase activity in the more concentrated homogenates. An alternative interpretation could be that DDT was bound to nonenzymic material in the homogenate. Ludwig & Barsa (61) found that $10^{-3}M$ DDT completely inhibited succinate oxidation in homogenates of eggs of *T. molitor* at the end of embryonic development.

Sacklin, Terriere & Remmert (79) made the striking observation that the oxidation of several Krebs-cycle intermediates was inhibited when DDT, at a concentration of $5 \times 10^{-6}M$, was added to a subcellular particulate preparation from *M. domestica*. Oxidative phosphorylation was concurrently reduced but there was no evidence of uncoupling. Unfortunately, the effects of noninsecticidal derivatives such as DDE were not studied. Winteringham has reported (97) a significant fall in the level of ATP of *M. domestica* adults *in vivo* which was not explained by the exhaustion of endogenous reserves as indicated by total oxygen consumption. In an experiment with DDT-treated *Calliphora erythrocephala* (Meigen), the thoraces were homogenized at a stage when the rate of oxygen consumption of the treated flies had fallen below that of the control and their total oxygen consumption did not exceed that of the control. In the absence of added substrate, the rate of oxygen uptake of the homogenate prepared from the treated flies was about 37 per cent of that of the control, whereas the rate of oxidation of added succinate was 40 per cent higher than in the control. (S. E. Lewis, G. M. Price & F. P. W. Winteringham, unpublished data). Tomizawa & Fukami (90) reported that DDT reduces nucleotide levels in nerve and muscle of intact *P. americana*. Gonda, Traub & Avi-Dor (37) found that DDT at a concentration of $10^{-4}M$ slightly reduced the oxidation of α-ketoglutarate and apparently uncoupled phosphorylation in a sarcosome preparation from *Aedes aegypti* (Linnaeus). At a concentration of $3 \times 10^{-4}M$, oxidation as well as phosphorylation was markedly reduced. More recent work by Avi-Dor (personal communication) suggests that similar effects

may be obtained with noninsecticidal derivatives and analogues of DDT.

Neuromuscular disturbances.—The unstabilizing effects of DDT on the nervous tissue of insects are well established. The signs *in vivo* are hyperexcitability of nerve and muscle, and there have been many attempts to locate these effects with semi-isolated nerve-muscle preparations. As a result of such studies with *P. americana* and with the frog, Dresden (26) concluded that DDT acts on the synapses in the nervous system, where it first produces facilitation and then block. On the other hand, Roeder & Weiant (78), working with a similar preparation from the roach, concluded that DDT acts on sensory receptors in the leg and produces trains of impulses in the afferent fibres of the crural nerve. Dresden's experiments were performed with preparations from roaches which had been injected with DDT and had developed symptoms before being dissected, whereas Roeder & Weiant introduced DDT after dissection. Since these reports appeared, Sternberg & Kearns (87) have shown that toxic substances can accumulate in the blood of the DDT-poisoned roach, and it is possible that such substances were present in Dresden's preparations. Yamasaki & Ishii (105) found that the "short trains" of impulses in the crural nerve of the DDT-poisoned cockroach were still present when, on being moved to a higher temperature, the insect recovered from locomotor instability.

Yamasaki & Narahashi (106) have recently attempted to characterize some biochemical lesion associated with the action of DDT *in vitro* on abdominal nerve-cord preparations from *P. americana* and *Periplaneta fuliginosa* (Serville). The potential difference between an uninjured central region of the ventral nerve cord and a cut peripheral end, soaked in Ringer's solution, was measured in different circumstances. The resting potential fell rapidly under conditions of anoxia but recoverd rapidly in the presence of oxygen. Iodoacetate and cyanide caused depolarization and falls in the resting potentials. DDT and its *p, p'*-alkoxy analogues caused slight depolarization and falls in the resting potentials only when the nerve was first soaked in Ringer's solution containing the insecticide at a concentration of $5 \times 10^{-5} M$. Under these conditions, DDT also slowed down the rate of repolarization following an electrically induced depolarization. It was suggested that the DDT effects might be caused physicochemically by its action on the nerve-membrane permeability to cations such as sodium and potassium or by interfering with the basal metabolism which normally maintains the resting potential of the nerve. In later experiments (107) Yamasaki & Narahashi studied the effects of iodoacetate, fluoride, and potassium ions, as well as rotenone, and DDT on the action potentials measured on the isolated nerve cord *in vitro*. The resting potential was not affected in most cases at a concentration of $1.4 \times 10^{-4} M$ DDT and conduction block was never observed. The negative afterpotential observed might be "caused by a stimulating action of DDT on the active metabolism of the nerve." Becht (7) has compared the effects of DDT and γ-BHC on the chordotonal organs (stretch sensory receptors) in the second thoracic leg of *P. americana*. When γ-BHC was injected into the intact insect, the sensory organs were unaf-

fected, despite the onset of paralysis and inexcitability. DDT, on the other hand, caused the trains of high-frequency action potentials observed by earlier workers (76, 78). These observations provide further evidence of the peripheral effects of DDT compared with the essentially central nervous effects of γ-BHC, [see review by Winteringham & Barnes (100)].

The mechanism by which DDT and its analogues unstabilize nervous tissue is far from clear. Attempts to differentiate between physical and biochemical mechanisms are beset by the difficulty of differentiating between biochemical effects produced by the direct action of the insecticide and those produced indirectly as consequences of some physical action. Several workers have suggested that DDT owes its action primarily to its physical properties, and that it produces disturbances in calcium or magnesium metabolism (77, 38). Hypotheses of narcotic action recently extended by Mullins (67, 68) suggest that DDT and γ-BHC may distort the lipoprotein macromolecular structure of the unspecified cell wall, so that the critical distribution of ions may be upset. Kearns has pointed out (52) the difficulty of testing this hypothesis, and there is an urgent need for study of the effects of DDT on the cytological distribution of metal ions and, indeed, of other substances, such as ACH, which may be involved in nerve metabolism.

Winteringham *et al.* (101) have made some exploratory experiments on the possible effects of DDT on the intra- and extracellular sodium/potassium ratios by an indirect technique. Sodium-24 and potassium-42 mixtures were injected into control and DDT-treated adult *M. domestica,* and the distributions of sodium and potassium between the head, thorax, and abdomen determined. The results indicated that DDT affected the over-all body distribution of sodium and potassium, in a way consistent with a reduction in cell-wall permeability to sodium or potassium. Later attempts to demonstrate corresponding changes in the sodium/potassium ratios in the blood of the wings were not conclusive (Winteringham *et al.*, unpublished data). Tobias (88) was unable to find any change in nerve-cord potassium of *P. americana* poisoned with DDT.

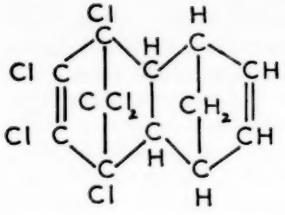
Tobias, Kollros & Savit (89) reported an increase in the level of ACH in *M. domestica* and in the nerve cord of *P. americana* when the insects reached "the prostrate, practically immobile stage" after treatment with DDT. Lewis (57) showed that ACH is synthesised in homogenates of whole flies prepared in eserinised Ringer's solution, as described by Tobias *et al.* Furthermore, the rate of synthesis is increased in homogenates prepared from DDT-poisoned flies at the prostrate stage. When ACH is extracted by methods which eliminate synthesis and hydrolysis (31, 59), there is no change in the level of ACH in DDT-treated flies at the prostrate stage (32). In the cockroach nerve cord the increase in ACH has been confirmed, and there is, moreover, a small increase in the ACH content of the thorax of the DDT-poisoned male roach before the insect is prostrate (K. S. Fowler & S. E. Lewis, unpublished observations). On the other hand, Colhoun (23) was unable to find any abnormal amount of ACH in the thoracic ganglia of the DDT-poisoned roach at prostration.

Lethal action.—The presence of DDT in the insect unstabilizes the nervous system and thus causes hyperneuromuscular activity. There is evidence that this activity is associated with biochemical disturbances which result in the accumulation of compounds such as ACH and the loss of high-energy phosphorus compounds. Whether the biochemical effects are causes of the nervous instability or the consequences of some physical action such as postulated by Mullins (67, 68) has yet to be established. Florey (28, 29) suggested that the sensory nerves of arthropods produce an "actions-substance" which is split by a specific enzyme, and that this enzyme is inhibited by DDT. In this connection, Sternburg, Chang & Kearns (86) suggest that the toxin found in DDT-poisoned roaches may normally be involved in the transmission of nerve impulses: "During DDT poisoning the toxin may accumulate in the blood and disrupt the entire nervous system." The same workers have also shown that the toxin can accumulate as a result of mild electrical stimulation. Thus, although accumulation of this toxin may result in the death of the insect, its formation would appear to be the result, rather than the cause, of the initial DDT symptoms.

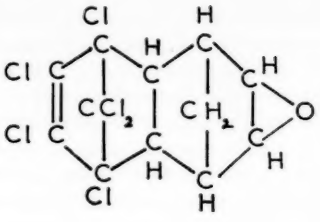
The suggestion that ACH may be released by DDT was put forward by Tobias *et al.* (89), and, while some of their results have not been confirmed, the idea still seems to be plausible. The fact that during the hyperactive stage there is no increase in the amount of ACH in insects does not invalidate the suggestion, since ACH is unlikely to accumulate at the site at which it is normally released, unless cholinesterase is inhibited. The turnover of ACH, however, may be markedly increased, but there is still no indication of the way in which DDT may produce this or the other effects associated with its action.

Aldrin and γ-BHC Group of Insecticides

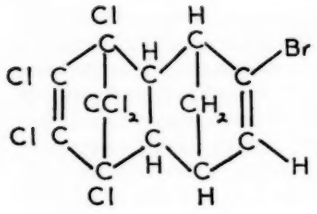
Common mechanism of action.—Busvine (14) has drawn attention to a similarity in the spectra of tolerance of four strains of adult *M. domestica* resistant to a group of insecticides consisting of α-chlordane, β-chlordane, isodrin, endrin, γ-BHC, aldrin (I) and dieldrin (II) but not to DDT. The similarity of the signs of poisoning by the chlordane-aldrin group, such as the "fanning" movements of the wings, was also pointed out. Busvine further suggested that all the compounds of this group "have similar insecticidal action by reason of similar toxaphores," and his study of molecular models indicated that the toxaphore is a pentagonal arrangement of chlorine atoms. Busvine's argument has provided a stimulating and rational attempt to explain not only the similar action of this group of insecticides including γ-BHC but also the phenomenon of cross resistance. Recent data, however, reveal a serious difficulty. Compounds III and IV were found to be relatively noninsecticidal against the adult housefly (F. P. W. Winteringham & A. Harrison, unpublished data). But compounds V and VI are highly toxic (E. C. H. Kolvoort, personal communication on behalf of Shell Research Ltd.). Thus, there are compounds (III and IV) closely related to aldrin and dieldrin (I and II)



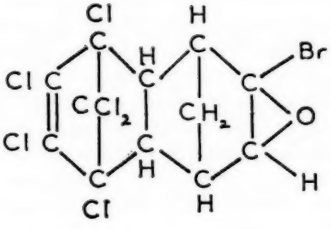
I



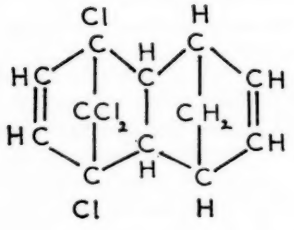
II



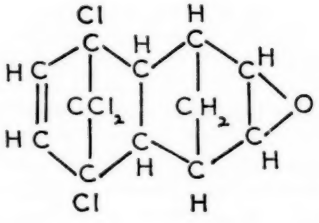
III



IV



V



VI

Derivatives of Aldrin (I)

which possess the pentagonal arrangement of chlorine atoms and yet are noninsecticidal, and similar compounds (V and VI) not possessing the pentagonal arrangement of chlorine atoms which are highly insecticidal.

Neurophysiological disturbances.—Surprisingly little advance has been made in the understanding of the mode of action of this important group of insecticides. Recent work has tended to confirm earlier indications of their essentially central nervous action in insects and mammals.

Gowdey & Stavraky (39) have compared the peripheral parasympa-

thomimetic action of aldrin and dieldrin in vagotomised and adrenalectomised cats under chloralose and urethane anaesthesia. Shortly after injection, aldrin slowed the heart and apparently affected the acetylcholinesterase activity of the blood. It had earlier been shown that aldrin potentiated and prolonged the effects of acetylcholine on the mammalian vagus. These peripheral effects were not observed in the case of dieldrin in spite of its central action. This reported difference is surprising, because there is evidence that aldrin is enzymically converted into dieldrin in mammals (J. M. Barnes, personal communication) and in insects [(36) and R. B. March, R. L. Metcalf & A. Baich, 1957, personal communication]. Isodrin is similarly converted to endrin in the adult housefly (G. T. Brooks, unpublished data). Giannotti, Metcalf & March (36) have studied the action of aldrin and dieldrin in *P. americana*. They found these compounds to be of very similar toxicity whether applied topically or injected, which strongly suggested rapid absorption of these compounds through the integument. They concluded that aldrin and dieldrin act centrally, the symptoms being antagonised by the action of nicotine. Unlike DDT, neither aldrin nor dieldrin produced symptoms in isolated legs.

Becht (7) has compared the effects of DDT and γ-BHC on the chordotonal organs of *P. americana*. Injected DDT resulted in the characteristic trains of high-frequency action potentials, while the sensory organs were unaffected by γ-BHC, despite the onset of paralysis. Yamasaki & Narahashi (108) have studied the action of dieldrin on the nervous tissue of *P. americana*. They concluded that dieldrin augmented the spontaneous activity of the nerve cord and in this respect resembled γ-BHC. Furthermore, γ-BHC and dieldrin did not induce locomotor instability resulting from excitability of sensory and motor neurones.

Lethal action.—The available evidence mainly indicates that the insecticides of this group have a similar mode of action and do not induce the peripheral nervous effects characteristic of DDT. There is no final evidence of an effect on a specific enzyme. F. P. W. Winteringham & A. Weatherley (unpublished data) observed that the respiration of dieldrin-poisoned *M. domestica* fell off far more rapidly *in vivo* than would be explained by the exhaustion of endogenous reserves. Morrison & Brown (66) found that aldrin, dieldrin, and γ-BHC caused slight inhibition *in vitro* of cytochrome oxidase prepared from the leg muscle of *P. americana*, but the effects were not characteristic and were shown by a number of unrelated insecticides.

This again calls attention to the attractive and stimulating hypothesis developed by Mullins (68) to account for the action of DDT and γ-BHC (see above). Other molecules, such as the noninsecticidal isomers of BHC, may gain access to the protein molecule interspaces, but their juxtaposition causes narcosis merely by blocking, not by distorting the interspace.

As Kearns (52) has pointed out, this interesting hypothesis raises

several questions. As already discussed, the action of γ-BHC resembles that of the cyclodiene derivatives rather than that of DDT, even with respect to its action on the same nervous organ, so that a common structural factor in the molecules of the γ-BHC-aldrin group must be sought to account for the distorting properties. Presumably, this common factor is not the pentagonal arrangement of chlorine atoms described by Busvine (see above). A second difficulty is that if the distortion caused by DDT and γ-BHC results in the leakage of a common ion, even at different rates, the resulting neurophysiological disturbances might be expected to be similar. In fact, there are fundamental qualitative and quantitive differences, and a specific ion leakage at the height of nervous excitation remains to be demonstrated. An experimental demonstration of this might be complicated by the possibility of a critical redistribution of potassium, for example within the Hoyle sheath, without there being any effect on blood potassium.

There is, nevertheless, reason to believe that the insecticidal actions of DDT and the γ-BHC groups are due to their primary physical action on nervous tissue. They may so distort or damage the nerve cell or axon wall that uncontrolled loss of ions or metabolic regulators results. There is evidence that the level of ACH in the nerve cord of *P. americana* may be increased by the action of DDT (89, 32) and γ-BHC (89); possibly there is uncontrolled release of "bound" ACH normally held in reserve for synaptic transmission and normally limiting its own synthesis. The release of metabolic regulators in this way may also be associated with the appearance of toxins in the blood of poisoned insects (87, 80, 12).

That these toxins may, however, be nonspecific consequences of increased nervous activity is supported by evidence of their appearance in the blood as a result of the action of apparently unrelated insecticides such as DDT (87), pyrethrins (12), TEPP (23) and as a result of exhaustion-induced paralysis (6) and electrical stimulation (86). The loss of metabolic regulators or coenzymes from mitochondria might similarly account for the premature loss of ATP in both nerve and muscle tissue of DDT-treated insects (see above).

Organophosphorus Insecticides

The importance of organophosphorus compounds as chemical-warfare agents has stimulated a tremendous amount of research on their mode of action and on the development of antidotes. Any detailed discussion of recent developments in this field is, therefore, beyond the scope of the present review. Instead, certain aspects of their action in insects will be mentioned because of their possible significance in selective insecticidal action.

There is abundant evidence of the powerful inhibitory properties of the insecticidal organophosphorus compounds or their metabolites against esterases (1, 15). Much is known about the mechanisms of inhibition of the cholinesterases, and there is little doubt that the inhibition of acetyl-

cholinesterase by organophosphorus compounds is closely associated with their insecticidal action (64, 84, 35). Recent work has tended to examine the biochemical and physiological consequences of cholinesterase inhibition in insects in an attempt to establish whether this inhibition is the sole toxic mechanism.

Occurrence and role of acetylcholine in insect nervous tissue.—There is now little doubt that acetylcholine occurs in insect tissues and in nervous tissues especially (4, 20, 21, 33, 57, 59, 65, 81, 102, 109). Furthermore, ACH synthesis has been demonstrated in the nerve cord from *P. americana* (89) and in flies (57). A detailed study by Smallman (81) of ACH synthesis in *Lucilia sericata* (Meigen) has shown that the enzyme systems involved resemble, in many respects, those found in vertebrate brain and that they are present in very high concentrations. Thus it would seem that ACH may be playing an important role in the metabolism of the insect nervous system. Nevertheless, its role in synaptic transmission and neuromuscular processes remains to be demonstrated unequivocally. Twarog & Roeder (92) investigated the action of various pharmacological agents, including ACH, on the last abdominal ganglion of *P. americana* from which the Hoyle sheath (48, 91) had been removed to facilitate access of the test substance. The preparation was continuously perfused with saline to which the test substance could be added without interruption. Postsynaptic responses resulting from electrical stimulation of the cercal nerve were studied in untreated and treated preparations. It was confirmed that the protective outer sheath of the cockroach central nervous system was responsible for the earlier observations on the ineffectiveness of ACH applied directly to the protected ganglion (91). At the same time, surprisingly high concentrations of ACH ($10^{-3}M$) were required to produce synaptic response and block, and Twarog & Roeder concluded that "the specificity of the synaptic action of acetylcholine remains in question."

Harlow (41) applied various substances to an isolated leg preparation from the locust and to a similar preparation which included the metathoracic ganglia. ACH was without effect on either preparation, and it was concluded that "there is no evidence for a cholinergic mechanism at the neuromuscular junction in the leg muscles," and that "the association of acetylcholine with synaptic function in the central nervous system has yet to be demonstrated in insects." ACH was without effect also when it was directly injected through the ganglion protective sheath.

While the inhibitory effects of the organophosphorus compounds on insect cholinesterase *in vivo* and *in vitro* are beyond doubt, van Asperen (3) and Rasmuson & Holmstedt (75) have shown that some estimates of the *in vivo* inhibition of cholinesterase may be too high, since more cholinesterase may become available to the anticholinesterase when the tissue is homogenized. This would result in a much greater inhibition in the homogenate than that obtaining *in vivo*. It is only recently that studies of the effects on the metabolism of ACH itself have been made.

The importance of using extraction techniques which preclude changes in ACH content has been amply demonstrated (59, 31).

Winteringham & Harrison (102) have studied the effects of topically applied DFP on the level of (C^{14}) ACH in the head of adult *M. domestica* labelled *in vivo* by the prior injection of (2-C^{14}) acetate. DFP caused a temporary rise in the level of (C^{14}) ACH, but the level fell off to a normal value long before the death of the insect. This suggested a similar pattern of change in the level of total ACH. Lewis & Fowler (58) showed that when DFP was applied topically in lethal amounts to adult *Calliphora erythrocephala* (Meigen), there was only a very slight increase in the level of ACH in the head tissues. It was followed, however, by a marked fall. There was a corresponding rise in the total ACH content of the abdomen. These workers concluded that DFP mainly caused a redistribution of ACH between the head and the abdomen.

Smallman & Fisher (82) have reported that the level of ACH rose in the head of adult *M. domestica* exposed to TEPP and then fell. Moreover, these workers were able to show that the rise and fall in ACH roughly coincided in time with the inhibition and spontaneous reactivation of cholinesterase. They also claimed that the duration of ACH accumulation was related to the death or survival of the insect.

Colhoun (23) studied the effect of TEPP on the ACH content of the thoracic nerve cord of male *P. americana*. He found a small temporary increase after ½ hr. which coincided with a period of nervous hyperactivity of the intact roach. There was a delayed but very much greater increase over the succeeding 24 hr. when, however, the nerve cord was electrically dead.

Notwithstanding the precise role of ACH in insect neuromuscular function, it is clear that organophosphorus compounds not only inhibit cholinesterase *in vivo* but may affect the turnover of ACH in nervous tissue. Further data are urgently required on the neurophysiological significance of these effects.

It may seem surprising that the remarkably rapid turnover of ACH does not result in a much more spectacular accumulation in the face of anticholinesterase action *in vivo*. On the other hand, ACH synthesis in the intact insect may be limited indirectly by the concentration of ACH held by intracellular particles at the synapse (43). Thus DFP, which certainly induces synaptic block (92) and paralysis of the intact insect, also markedly reduced the rate of (C^{14}) acetate incorporation into ACH in the head tissues of adult *M. domestica* (103), although presumably there would be no direct effect on the choline acetylase system (69). There is evidence that both in insects (24) and in mammals (93) ACH may, indeed, be stored in intracellular particles.

Lethal action.—There is no doubt that a major biochemical lesion in insects exposed to lethal doses of organophosphorus compounds is the inhibition of acetylcholinesterase and the consequent disturbances in the metabolism of the substrate. Organophosphorus compounds, however,

are potent phosphorylating agents and are capable of inhibiting many enzymes other than acetylcholinesterase (1, 35). It follows, therefore, that lesions other than acetylcholinesterase inhibition may contribute to the signs of poisoning and the ultimate death of the insect. There is recent evidence that in some cases these secondary lesions may be important. For example, although the enzymes associated with the metabolism of ACH appear at an early stage in the development of the insect embryo (109, 85), it has been suggested that the ovicidal properties of the organophosphorus compounds cannot be attributed to their action on acetylcholinesterase (85, 60, 74). Potter and his colleagues concluded that "whilst inhibition of cholinesterase may play a part in the poisoning process, at least under some conditions, the evidence indicates that the death of the embryo may result from some other causes." Smith & Wagenknecht (83), on the other hand, working with parathion, conclude that cholinesterase activity is inhibited when the enzyme appears in the developing embryo and that this inhibition is lethal at the last stage of embryonic development. These workers suggest that the concentrations of TEPP used by Lord & Potter (60) are excessive, "and may have introduced modes of action which do not occur under conditions of actual usage."

Harvey & Brown (42) observed that injected parathion and TEPP caused a marked increase in the respiration of adult male *Blattella germanica* (Linnaeus) during the hyperactive stage, but the respiration was reduced when the insects became paralysed. Winteringham and his colleagues (98, 102, 103), however, found a greatly increased oxygen uptake after the onset of paralysis as a result of exposure of adult *M. domestica* to DFP and to malathion (unpublished data), and there was also a delayed accumulation of glutamine. The acute and lethal effects of anticholinesterase action in mammals may be largely ascribed to the failure of the neuromusculature of respiration and the consequent anoxia (54, 5). This suggests some additional lesion in the insect, since there was no respiratory failure and it is difficult to explain the stimulated respiration of the paralysed insect in terms of cholinesterase inhibition. The glutamine accumulation may, however, be a nonspecific consequence of the stimulated respiration (99). Anticholinesterase action may be reversed *in vivo* and *in vitro* by certain hydroxylamine derivatives of which pyridine-2-aldoxime methiodide (PAM) is one of the most effective (22, 40, 47, 55, 56, 96), provided phosphorylation of the cholinesterase has not reached the second irreversible stage postulated by Hobbiger (44, 45). Mammals may, in fact, be protected from otherwise lethal doses of organophosphorus compounds by suitable injections of PAM (22, 47, 53, 55, 95), and the injection of PAM in combination with atropine is particularly effective (46, 95). J. M. Barnes (personal communication, 1957) observed that this combination protected rats from otherwise lethal doses of malathion. Neither PAM nor PAM and atropine protected adult *M. domestica* from the lethal effects of either DFP

or malathion, and the stimulated oxygen uptake was unaffected. In the case of DFP, there was a temporary recovery from the paralysis but the insects later suffered a relapse (98, 103). These observations strongly suggest an injurious lesion in insects in addition to that of cholinesterase inhibition. O'Brien (71) has estimated that no more than a 50-per-cent inhibition of the aromatic esterase attacking *o*-nitrophenyl acetate is caused in adult *P. americana* by a lethal dose of TEPP. Van Asperen (3), on the other hand, found that at the stage of knockdown of adult *M. domestica* by DDVP the aliesterase attacking methyl butyrate was more strongly inhibited than the acetylcholinesterase, and suggested that this was "evidence against an essential role of cholinesterase inhibition in the insecticidal action of DDVP." O'Brien (72) observed that pyruvate and citrate oxidation was inhibited *in vitro* by malathion in mouse brain and in cockroach thoracic preparations, but found little effect either on the oxidation of other compounds of the tricarboxylic-acid cycle or on the glycolytic system. He suggested that interference with carbohydrate metabolism was not likely to be connected with the insecticidal action of malathion, and he later concluded that this compound behaved as a "typical organophosphorus insecticide (73)." Winteringham and his colleagues [(102) and unpublished data] found that DFP had no significant effect on the phosphorus metabolism of the adult house fly, even at the late stage of prostration.

In conclusion to this section, it may be stated that although organophosphorus compounds inhibit acetylcholinesterase and this probably accounts for the hyperactivity and subsequent paralysis of the insect, the stimulated oxygen consumption, failure of the protective action of PAM and atropine combinations, and the inhibition of other esterases indicate that other lesions can occur. In certain cases these secondary lesions may be very important. For example, Colhoun (23) found that the blood of roaches poisoned with TEPP contains a substance which stimulates the nerve cord of an untreated roach, the presence of such a substance would probably produce secondary effects other than those usually associated with the inhibition of cholinesterase.

Miscellaneous Insecticides

SH-enzyme inhibitors.—Attention has recently been turned to the insecticidal action of the halogenated acetic acid derivatives of which iodoacetate is the classical example. The injection of lethal doses of iodoacetate into adult house flies has been found to cause a marked depletion of phosphoglycerate at the stage of prostration (104). Respiration was also reduced to about one third of its normal level but continued for several hours, despite the immobility of the insect (F. P. W. Winteringham & A. Weatherley, unpublished data). The observed phosphoglycerate depletion *in vivo* is an expected consequence of triosephosphate dehydrogenase inhibition and is consistent with the observations of Bettini & Boccacci (8, 9, 10, 11). They were able to relate the mortality in insects

treated with iodoacetate to the inhibition of triosephosphate dehydrogenase, *in vivo* and *in vitro*, but not to the inhibition of succinic dehydrogenase or to the blocking of coenzyme-A function. Chefurka, on the other hand, reported that triosephosphate dehydrogenase in insects is relatively insensitive to iodoacetate (16). The continued residual respiration of the iodoacetate-poisoned insect may be caused by the operation of the hexose shunt, which is relatively insensitive to SH-enzyme inhibitors (27). The hexose shunt has been demonstrated in the adult house fly by Chefurka (17, 18, 19). Low doses of methyl bromide, another SH-enzyme inhibitor, may, however, exert their lethal action in the insect by some mechanism other than the inhibition of triosephosphate dehydrogenase (104).

Forgash (30) has demonstrated a fall in the level of reduced glutathione in the muscle and fat body of *P. americana* as a result of arsenic trioxide injection. The female insect tolerated higher doses of this compound than did the male, and it was suggested that the larger amount of reduced glutathione in the female is responsible for this difference.

Botanicals and synthetic relatives.—Blum & Kearns (12) have shown that pyrethrum poisoning in *P. americana* was associated with the appearance of a toxin in the blood and that this toxin was not a pyrethroid metabolite. Naidu & Zaheer (70) studied the action of allethrin on the isolated heart of *P. americana*. They suggested that the action of allethrin was due to the release of ACH at the neuromuscular junctions. Fukami & Tomizawa (34) concluded that the inhibitory action of rotenone on the respiratory metabolism of nerve and muscle resulted from inhibition of L-glutamic acid dehydrogenase.

Concluding Comments

We conclude our argument that lethal action is probably the result of a combination of lesions by a brief summary of the principal evidence:

DDT and its insecticidal relatives unstabilize nervous tissue by some physical mechanism which appears to be associated with the liberation of acetylcholine and/or other toxins. There is evidence of effects on oxidative metabolism which are not merely consequences of the neurophysiological disturbances.

Organophosphorus compounds clearly inhibit enzymes in addition to acetylcholinesterase. Other effects—such as the stimulated oxygen consumption, liberation of toxins other than acetylcholine, the temporary or ineffective nature of PAM and atropine in reversing the action of anticholinesterases—are not readily explained in terms of acetylcholinesterase inhibition.

The inhibition of insect glutamic acid oxidation by rotenone has been reported. This effect is unlikely to be related to nervous disturbances implied by the great sensitivity of the insect heart to this insecticide, as shown some years ago by Krijgsman and his colleagues (56a).

There is abundant evidence that contact insecticides penetrate the insect cuticle much more rapidly than the mammalian skin (98).

Observations such as these suggest to us that effective insecticidal action is not so much a function of some unique property of the molecule as a unique combination of properties, none of which need be specific for the molecule per se.

LITERATURE CITED

1. Aldridge, W. N., *Ann. Repts. on Progr. Chem. (Chem. Soc. London)*, **53**, 294–305 (1956)
2. Anderson, A. D., March, R. B., and Metcalf, R. L., *Ann. Entomol. Soc. Am.*, **47**, 595–602 (1954)
3. Asperen, K. van., *Nature*, **181**, 355–56 (1958)
4. Augustinnson, K. B., and Grahn, M., *Acta Physiol. Scand.*, **32**, 174–90 (1954)
5. Barnes, J. M., *Chem. & Ind. (London)*, 478–80 (1954)
6. Beament, J. W. L., *J. Insect Physiol.*, **2**, 199–214 (1958)
7. Becht, G., *Nature*, **181**, 777–79 (1958)
8. Bettini, S., and Boccacci, M., *Rend. ist. super. sanità*, **17**, 188–91 (1954)
9. Bettini, S., and Boccacci, M., *Experientia*, **11**, 70–71 (1955)
10. Bettini, S., and Boccacci, M., *Papers ist. super. sanità*, **1**, 155–75 (1956)
11. Bettini, S., and Boccacci, M., *J. Econ. Entomol.*, **49**, 554–55 (1956)
12. Blum, M. S., and Kearns, C. W., *J. Econ. Entomol.*, **49**, 862–65 (1956)
13. Brown, B. E., and Brown, A. W. A., *J. Econ. Entomol.*, **49**, 675–79 (1956)
14. Busvine, J. R., *Nature*, **174**, 783–85 (1954)
15. Casida, J. E., *J. Agr. Food Chem.*, **4**, 772–85 (1956)
16. Chefurka, W., *Enzymologia*, **17**, 73–89 (1954)
17. Chefurka, W., *Biochim. et Biophys. Acta*, **17**, 294–96 (1955)
18. Chefurka, W., *Enzymologia*, **18**, 209–27 (1957)
19. Chefurka, W., *Can. J. Biochem. and Physiol.*, **36**, 83–102 (1958)
20. Chefurka, W., and Smallman, B. N., *Nature*, **175**, 946–47 (1955)
21. Chefurka, W., and Smallman, B. N., *Can. J. Biochem. and Physiol.*, **34**, 731–42 (1956)
22. Childs, A. F., Davies, D. R., Green, A. L., and Rutland, J. D., *Brit. J. Pharmacol.* **10**, 462–65 (1955)
23. Colhoun, E. H., *Science*, **127**, 25 (1958)
24. Colhoun, E. H., *Nature*, **181**, 490 (1958)
25. Dahm, P. A., *Ann. Rev. Entomol.*, **2**, 247–60 (1957)
26. Dresden, D., *Physiological Investigations into the Action of DDT* (G. W. van der Wiel & Co., Arnheim, Netherlands, 114 pp., 1949)
27. Dickens, F., and Glock, G. E., *Biochem. J.*, **50**, 81–95 (1951)
28. Florey, E., *Pflanzenschutz Ber.*, **6**, 134–52 (1951)
29. Florey, E., *Pflanzenschutz Ber.*, **7**, 81–141 (1951)
30. Forgash, A. J., *J. Econ. Entomol.*, **50**, 750–52 (1957)
31. Fowler, K. S., and Lewis, S. E., *J. Physiol.*, **142**, 165–72 (1958)
32. Fowler, K. S., and Lewis, S. E., *Pest Infestation Research 1957*, 37 (Her Majesty's Stationery Office, London, England, 55 pp., 1958)
33. Frontali, N., *J. Insect Physiol.*, **1**, 319–26 (1958)
34. Fukami, J., and Tomizawa, C., *Botyu-Kagaku*, **21**, 129–33 (1956)
35. Fukuto, T. R., *Advances in Pest Control Research*, **1**, 147–93 (1957)

36. Giannotti, O., Metcalf, R. L., and March, R. B., *Ann. Entomol. Soc. Am.*, **49**, 588–92 (1956)
37. Gonda, O., Traub, A., and Avi-Dor, Y., *Biochem. J.*, **67**, 487–93 (1957)
38. Gordon, H. T., and Welsh, J. H., *J. Cellular Comp. Physiol.*, **31**, 395–419 (1948)
39. Gowdey, C. W., and Stavraky, G. W., *Can. J. Biochem. and Physiol.*, **33**, 272–82 (1955)
40. Green, A. L., and Smith, H. J., *Biochem. J.*, **68**, 28–35 (1958)
41. Harlow, P. A., *Ann. Appl. Biol.*, **46**, 55–73 (1958)
42. Harvey, G. T., and Brown, A. W. A., *Can. J. Zool.*, **29**, 42–53 (1951)
43. Hebb, C. O., *Physiol. Revs.*, **37**, 196–220 (1957)
44. Hobbiger, F., *Brit. J. Pharmacol.*, **11**, 295–303 (1956)
45. Hobbiger, F., *Brit. J. Pharmacol.*, **12**, 438–46 (1957)
46. Hobbiger, F., *Biochem. J.*, **66**, 7P (1957)
47. Holmes, R., and Robins, E. L., *Brit. J. Pharmacol.*, **10**, 490–95 (1955)
48. Hoyle, G., *Nature*, **169**, 281–82 (1952)
49. Johnston, C. D., *Arch. Biochem. Biophys.*, **31**, 375–82 (1951)
50. Judah, J. D., *Brit. J. Pharmacol.*, **4**, 120 (1949)
51. Judah, J. D., and Williams-Ashman, H. G., *Biochem. J.*, **48**, 33–42 (1951)
52. Kearns, C. W., *Ann. Rev. Entomol.*, **1**, 123–48 (1956)
53. Kewitz, H., *Arch. Biochem. Biophys.*, **66**, 263–70 (1957)
54. Kewitz, H., and Nachmansohn, D., *Arch. Biochem. Biophys.*, **66**, 271–83 (1957)
55. Kewitz, H., Wilson, I. B., and Nachmansohn, D., *Arch. Biochem. Biophys.* **64**, 456–65 (1956)
56. Koelle, G. B., *Science*, **125**, 1195–96 (1957)
56a. Krijgsman, B. J., Dresden, D., and Berger, N. E., *Bull. Entomol. Research*, **41**, 141–51 (1950)
57. Lewis, S. E., *Nature*, **172**, 1004 (1953)
58. Lewis, S. E., and Fowler, K. S., *Nature*, **178**, 919–20 (1956)
59. Lewis, S. E., and Smallman, B. N., *J. Physiol. (London)*, **134**, 241–56 (1956)
60. Lord, K. A., and Potter, C., *Chem. & Ind. (London)*, 1214–17 (1954)
61. Ludwig, D., and Barsa, M. C., *Ann. Entomol. Soc. Am.*, **50**, 475–77 (1957)
62. Ludwig, D., Barsa, M. C., and Cali, C. T., *Ann. Entomol. Soc. Am.*, **48**, 165–70 (1955)
63. Metcalf, R. L., *Physiol. Revs.*, **35**, 197–232 (1955)
64. Metcalf, R. L., *Organic Insecticides* (Interscience Publishers, Inc., New York, N.Y., 392 pp., 1955)
65. Metcalf, R. L., March, R. B., and Maxon, M. G., *Ann. Entomol. Soc. Am.*, **48**, 222–28 (1955)
66. Morrison, P. E., and Brown, A. W. A., *J. Econ. Entomol.*, **47**, 723–30 (1954)
67. Mullins, L. J., *Chem. Rev.*, **54**, 289–323 (1954)
68. Mullins, L. J., *Science*, **122**, 118–19 (1955)
69. Nachmansohn, D., and Wilson, I. B., *Advances in Enzymol.*, **12**, 323 (1951)
70. Naidu, M. B., and Zaheer, S. H., *J. Entomol. Soc. India*, **18**, 57–62 (1956)
71. O'Brien, R. D., *Ann. Entomol. Soc. Am.*, **50**, 223–29 (1957)
72. O'Brien, R. D., *J. Econ. Entomol.*, **50**, 79–84 (1957)
73. O'Brien, R. D., *J. Econ. Entomol.*, **50**, 159–64 (1957)
74. Potter, C., Lord, K. A., Kenten, J., Salkeld, E. H., and Holbrook, D. V., *Ann. Appl. Biol.*, **45**, 361–75 (1957)

75. Rasmuson, B., and Holmstedt, B., *Kgl. Lantbruks-Högskol. Ann.*, **24**, 89–100 (1958)
76. Roeder, K. D., *Ann. Rev. Entomol.*, **3**, 1–18 (1958)
77. Roeder, K. D., and Weiant, E. A., *Ann. Entomol. Soc. Am.*, **44**, 372–80 (1951)
78. Roeder, K. D., and Weiant, E. A., *J. Cellular Comp. Physiol.*, **32**, 175–86 (1948)
79. Sacklin, J. A., Terriere, L. C., and Remmert, L. F., *Science*, **122**, 377–78 (1955)
80. Shankland, D. L., *Dissertation Abstr.*, **16**, 366 (1956)
81. Smallman, B. N., *J. Physiol. (London)*, **132**, 343–57 (1956)
82. Smallman, B. N., and Fisher, R. W., *Intern. Congr. Entomol., 10th Meeting* (Montreal, Que., Canada, 1956)
83. Smith, E. H., and Wagenknecht, A. C., *J. Econ. Entomol.*, **49**, 777–83 (1956)
84. Spencer, E. Y., and O'Brien, R. D., *Ann. Rev. Entomol.*, **2**, 261–78 (1957)
85. Staudenmayer, T., *Z. vergleich. Physiol.*, **37**, 416–23 (1955)
86. Sternburg, J., Chang, C. S., and Kearns, C. W., *Federation Proc.*, **16**, 124–25 (1957)
87. Sternburg, J., and Kearns, C. W., *Science*, **116**, 144–47 (1952)
88. Tobias, J. M., *J. Cellular Comp. Physiol.*, **31**, 125–41 (1948)
89. Tobias, J. M., Kollros, J. J., and Savit, J., *J. Cellular Comp. Physiol.*, **28**, 159–82 (1946)
90. Tomizawa, C., and Fukami, J., *Botyu-Kagaku*, **21**, 133–39 (1956)
91. Twarog, B. M., and Roeder, K. D., *Biol. Bull.*, **111**, 278–86 (1956)
92. Twarog, B. M., and Roeder, K. D., *Ann. Entomol. Soc. Am.*, **50**, 231–37 (1957)
93. Whittaker, V. P., *Biochem. J.*, **68**, 21P (1958)
94. Wigglesworth, V. B., *Das Insektizid Dichlorphenyl-trichloräthan und seine Bedeutung*, 93–111 (Birkhäuser, Basel, Switzerland, 299 pp., 1955)
95. Wills, J. H., Kunkel, A. M., Brown, R. V., and Groblewski, G. E., *Science*, **125**, 743–44 (1957)
96. Wilson, I. B., Ginsberg, S., and Meislich, E. K., *J. Am. Chem. Soc.*, **77**, 4286–91 (1955)
97. Winteringham, F. P. W., *Chem. & Ind. (London)*, 1182–86 (1956)
98. Winteringham, F. P. W., *Chem & Ind. (London)*, 1195–1202 (1957)
99. Winteringham, F. P. W., *Intern. Congr. Biochem., 4th Meeting* (Vienna, Austria, 1958)
100. Winteringham, F. P. W., and Barnes, J. M., *Physiol. Revs.* **35**, 701–39 (1955)
101. Winteringham, F. P. W., Drysdale, S., Hallowes, K. H., and McKay, M. A., *Pest Infestation Research 1956*, 46–50 (Her Majesty's Stationery Office, London, England, 64 pp., 1957)
102. Winteringham, F. P. W., and Harrison, A., *Nature*, **178**, 81–83 (1956)
103. Winteringham, F. P. W., Harrison, A., McKay, M. A., and Weatherley, A., *Biochem. J.*, **66**, 49P–50P (1957)
104. Winteringham, F. P. W., Hellyer, G. C., and McKay, M. A., *Biochem. J.*, **69**, 640–48 (1958)
105. Yamasaki, T., and Ishii, T., *Botyu-Kagaku*, **19**, 1–14 (1954)
106. Yamasaki, T., and Narahashi, T., *Botyu-Kagaku*, **22**, 259–77 (1957)
107. Yamasaki, T., and Narahashi, T., *Botyu-Kagaku*, **22**, 354–67 (1957)
108. Yamasaki, T., and Narahashi, T., *Botyu-Kagaku*, **23**, 47–54 (1958)
109. Yushima, T., *J. Econ. Entomol.*, **50**, 440–43 (1957)

BIOLOGICAL ASSAY OF INSECTICIDE RESIDUES[1]

By Sumio Nagasawa
Institute for Chemical Research, Kyoto University, Takatsuki, Japan

Recently, Gunther & Blinn (55) have described the characteristics of insecticide residues and have given a definition which differentiates them from insecticide deposits. They used the word "deposit" for insecticide initially laid on a surface by treatment, and the word "residue" for insecticide, regardless of its locale on or within a substratum, which has in any way aged. If there is some interval between the time of the application and the time the insecticide reaches the insect, so that weathering, metabolic degradation, and other processes take place, the deposit becomes a residue. Thus, all kinds of stomach poisons are used as a form of residue. With systemic insecticides also, there is some lapse of time before the active ingredients reach the insects through the plant tissues. Contact insecticides which are used so as to come in contact with insects after having been sprayed on the substratum, not directly on the insects, must also be considered residues. Therefore, in a broad sense, we can say that insecticides used in forms other than residue are rather scarce and that the description of the method of biological assay of insecticide residues is tantamount to that of the biological assay of all insecticides. It is consequently very difficult to discuss it within limited pages. Fortunately, Sun (119) has recently reviewed in detail the same problem. Therefore, the present writer wishes to review in this paper the laboratory methods for the assay of insecticides which, after spraying, remain in a very minute amount as the result of the various environmental factors. Accordingly, the review of the results of practical field experiments will be omitted.

With some chemicals used as insecticides, such as pyrethrum, derris, cube, the active ingredients decompose very rapidly; on the other hand, for such chemicals as lead arsenate, which is very slow in its decomposition, appropriate physical or chemical methods for determination of minute amounts of residues have been developed. As long as insecticides were limited to these kinds, the problem of residues was not important. But the appearance of many effective organic insecticides, which followed the synthesis of DDT, led to difficulty in the detection of residues, for the several limited methods available were unsuitable. At the same time, the adoption of various auxiliary substances which prolong toxicity and, in addition, the prevalence of organic phosphorus insecticides which are highly toxic to men and cattle have made even a minute amount of residue an important subject of discussion.

There is no doubt that the importance of the problem of residues has

[1] The survey of the literature pertaining to this review was completed in January, 1958.

grown with the rapid increase in the use of insecticides, although, several chemical methods have been developed for determinations of small amounts of the residues of certain new synthetic insecticides. However, in cases where adequate chemical methods are lacking, various forms of biological assay can be used very profitably. A drawback of biological assay is non-specificity, but we can usually measure by this method a very small quantity of toxic residue with an accuracy unattainable by chemical methods.

Biological methods include tests of two main types. In the more commonly used method, graded doses of samples of unknown concentration are applied to a number of groups of animals such as living insects, small fishes, and ticks and the percentages of physiologically affected individuals so produced are compared with those produced by the application of doses of known concentrations of the pure chemical. The other type of test is biochemical, and it involves quantitative measurement of the insecticide or its derivative with isolated organs such as the muscles of the leech or the frog, cockroach nerve cords, or enzyme systems such as the cholinesterases or carbonic anhydrase. The review of biochemical measurements will be excluded from the present paper; the details of these methods are reviewed by Schechter & Hornstein (106). The author also wishes to call the reader's attention to two recent reviews concerned with techniques for testing insecticides, namely a general review by Busvine (20a) and one by Hoskins (59a) on bioassay in entomological research.

Residues on Substrata

The most common method for biological assay of residual insecticides involves the use of insecticidal films or deposits on test surfaces such as filter paper, cloth, glass plate, vials, plywood, wooden or metal plate, and other absorptive materials. Ordinarily, test insects are anesthetized lightly with ether or carbon dioxide or immobilized under a low temperature, then divided into groups of certain number and, if the situation demands it, sexed. After the insects have revived completely, they are exposed to test surfaces dipped in or sprayed with solutions of toxicant of known concentration in acetone, ether, ethanol, or benzol. Sometimes, the materials dissolved in acetone are applied with pipette to a piece of paper or woolen cloth. Eddy & Bushland (41) reported that a piece of woolen cloth took up an amount of liquid equal to its dry weight, so that the concentration of insecticide in an impregnated cloth was about the same as that in solution.

After treatment, the surface is allowed to dry and used for the test. At the end of the specified exposure period, the insects are transferred to a clean cage, provided with food, and held until mortality or knockdown counts are made. Checks can be obtained by subjecting the same number of test insects to the identical procedure except for the use of clean, untreated plates or to plates treated with solvent alone. The approximate LD_{50} is assessed with the aid of probit-log dosage paper. Of course, it is desirable to carry out the tests under constant environmental conditions.

It is important that the test insects be in contact with the treated sur-

face for the full test period. Dean (38) classified the film method into three categories: (*a*) discontinuous exposure, in which the insects are in contact with the film for a brief interval only, (*b*) continuous exposure, in which the insects are in contact with the sprayed surface for the whole test period, and (*c*) controlled exposure, in which insects are exposed for certain periods to the sprayed surface and then transferred to a clean cage. Doner (39) considered two test methods: that of free choice and that in which choice is not allowed. However, results obtained by Dahm & Pankaskie (33), Sun & Sun (117), and Sun & Pankaskie (118) on house flies and *Drosophila* showed that it is not necessary to cover all surfaces of the test jar with the toxicant. As long as the toxic residues were deposited in the same manner for both the standard and the treated samples, the final results did not vary appreciably. This may be due to the high activity of flies.

Impregnated paper method.—The early use of protective films of insecticide by Potter (101) in the control of insects was opportune, particularly in view of the later discovery of the insecticidal value of DDT, the outstanding feature of which is its effectiveness in the form of persistent residual films. Since the impregnated paper technique was first experimentally tested by Tattersfield & Potter (123) and Parkin & Green (96), its popularity has increased. Parkin & Green (97) published the first critical observation on the toxic property of DDT films, reporting that in certain circumstances toxicity was increased after the house fly, *Musca domestica* Linnaeus, had been once enclosed over it. Morrison (88) and Proverbs & Morrison (104) lined the inside of shell vials with filter paper impregnated with DDT or related compounds and enclosed *Drosophila melanogaster* Meigen in them. They studied the relative insecticidal activities of DDT and related organic molecules. On successive days of their embryonic development, Ludwig (82) placed the eggs of the Japanese beetle, *Popillia japonica* Newman, for 10 min. on a filter paper moistened with either a 5-per-cent or a 10-per-cent solution of DDT, and found that there was no effect on the embryonic development and no significant change in susceptibility of eggs with age. Although Busvine & Barnes (21) used papers impregnated with acetone solutions of DDT which gave dry deposits of the insecticides, their results were not entirely satisfactory because, as they pointed out, the evaporation of the volatile solvents leads to irregular size and distribution of DDT crystals, with consequent erratic results. Pradhan (102) investigated the concentration-time-mortality relations between DDT, γ-BHC, and adults of *Tribolium castaneum* (Herbst) and *Macrosiphoniella sanborni* (Gillette), and larvae of *Plutella maculipennis* (Curtis). He proved that *T. castaneum* is the most suitable of these for experimental laboratory work on films. Pradhan also described techniques for experiments in which attempts were made to eliminate the fumigation effect of γ-BHC, and he studied the effect of temperature on the toxicity of DDT films (103). Collins & Nardy (27) made a laboratory test to determine the residual effect of 5-per-cent DDT emulsion on larvae of *Dermacenter*

variabilis (Say), using pieces of bond typewriter paper. The paper with ticks was folded and placed in a muslin-covered quart jar. Fleming *et al.* (47), using filter paper moistened with dilute suspensions of insecticides, found that the response of *Macrocentrus ancylivorus* Rhower to DDT differed from its response to chlordane, and that males reacted faster than females when the sexes were exposed separately.

For the measurements of insecticidal potency to various insect pests, Busvine & Nash (22) dissolved samples of γ-BHC, dieldrin, aldrin, chlordane, DDT, toxaphene, Pyrolan, allethrin, and pyrethrin in refined mineral oil to prepare solutions of different concentrations, and the test filter papers were then impregnated with standard volumes of the solutions. The insects were confined on the treated filter papers under inverted glass funnels, except for the mosquitoes, which were enclosed in cylinders made by rolling up the filter papers and closing the ends with glass discs. In tests with γ-BHC, attempts were made to prevent undue concentration of the vapor. Thus, the insects were confined in glass rings instead of funnels and the ends of the paper cylinders used for mosquitoes were closed with mosquito netting instead of glass discs. Residual action was judged by the mortality of batches of bed bugs (*Cimex lectularius* Linnaeus) exposed to films of the insecticides, at intervals up to six months after preparation. The approximate order of persistence is: dieldrin = DDT = toxaphene > Pyrolan > γ-BHC = chlordane = aldrin > allethrin, pyrethrins. Oppenoorth & Dresden (93) introduced *D. melanogaster* into test tubes, the walls of which were covered with filter paper on which γ-BHC had been deposited. After a suspension of 50-per-cent DDT wettable powder was thoroughly stirred, Collins & King (28) poured an aliquot portion quickly onto a filter paper in a Büchner funnel and drew off the water by vacuum. The filter paper was taken out of the funnel and laid on a piece of clean muslin to dry, and finally put in a vial. Its outer edge was coated with vaseline, to attach the paper to the glass and confine the *Tribolium confusum* Duval to the treated surface. Collins & King concluded that under some conditions age of beetles and humidity may affect the results. For genetical studies of the DDT resistance of *Drosophila,* Crow (30) used a testing chamber consisting of a shell vial in which a piece of DDT-treated filter paper had been inserted so as to cover the inside surface, except the bottom and the cotton plug. The result of the experiments in which these areas were covered with DDT-treated paper showed no detectable difference in mortality from that of the above-described experiment. The data reported are all from females, for two reasons: the females seemed to give slightly more reproducible results, and in a subsequent test in which chromosome make-up was studied, the females lent themselves to a neater analysis because they have the same number of X chromosomes as autosomes. Bocking (10) and Merrell & Underhill (85) also used only females for the same reasons. Ishikura & Ozaki (63) examined the difference in the resistance to EPN of the cabbage armyworm, *Barathra brassicae* (Linnaeus), reared under different densities by this filter-paper method. The

results suggest the tolerance is primarily dependent on the body weight of the larvae, but that other factors may reduce it at high densities of rearing. Beran (8) and Beran & Neururer (9) exposed the honey bee, *Apis mellifera* Linnaeus, to insecticides deposited on a piece of round, thick filter paper, then enclosed it conically with cellophane paper. Utilizing the filter-paper technique developed by Parkin & Green (96), Hewlett & Gostick (58) studied the loss of weight of pyrethrin-treated red flour beetles (*T. castaneum*). The factors which affect the weight loss were dose, time of exposure, air movement, temperature, relative humidity, carrier oil, batch size, and method of preparation of films.

Davidow & Laug (37) developed a "masking technique" for bioassaying lindane deposits on paper, painted surfaces, cellophane, and linoleum. An aliquot of a surface is prepared for test by masking the remaining portion of the experimental area with Scotch tape. The method is based upon the principle that, within the limits of the assay, a quantity of insecticide distributed over a small area will result in the same percentage mortality to house flies as the identical quantity of insecticide spread over a larger area. Repeated assays result in the conclusion that the masking of portions of the total area is equivalent to taking aliquots of the dose within experimental variations.

Impregnated cloth method.—Instead of paper, various cloths have been used as the base of impregnation for the biological assay of insecticide residues. This technique has been applied especially in body-lice control and moth-proofing experiments. Bushland *et al.* (18, 19) placed the insecticide-impregnated disc of woolen cloth inside a beaker with adult body lice, *Pediculus humanus* Linnaeus. The treated cloth that allowed no normal survivors after 24 hr. was retested at various intervals to determine the duration of effectiveness until it no longer caused appreciable mortality. This method was used by Eddy & Carson (40) for measuring the knock-down caused by compounds toxic to body lice. When none of the insects could walk or cling to the pads, the interval from the beginning to that moment was noted as the knockdown time. Eddy & Bushland (41) reported that when the samples were evaluated by the modified beaker-test method, γ-BHC and technical chlordane were two of the most toxic. Both these materials were effective at a concentration of 0.0005 per cent. Chlorinated camphene and crude BHC were effective at 0.0025 per cent whereas DDT and 2-pivalyl-1, 3-indandione killed only a small number of the test insects at 0.005 per cent. Madden *et al.* (83) applied DDT and other insecticides to both sides of a circular woolen pad and about 1 hr. later dropped the pad into a half-pint fruit jar containing five normal adult bed bugs, *Cimex lectularis* Linnaeus. After 15 min., the pad was removed and the bugs were held in the jar for later mortality counts. For carpet-beetle tests, Ludwick & Decker (81) treated circular pieces of wool 2 sq. in. in area with 1 ml. of the alcohol solution of toxicant. After the alcohol evaporated from the wool, the circles were placed in 35 mm. crystallizing dishes and manually infested with 10 *Attagenus piceus* (Olivier) larvae.

In practice, cloth, cotton cords, screen strips, and strings impregnated with various chlorinated hydrocarbons or organophosphorus insecticides containing sugar or synergists have been used for the house-fly control in dairy barns and food shops. Data have been presented by several workers in this field (5, 70, 84, 98, 99, 107). Morris (87) writes on the persistence of toxicity in DDT-impregnated hessian and its use for control of tsetse flies, *Glossina palpalis* (Robineau-Desvoidy) and *Glossina tachinoides* Westwood. Treatment of cloth storage bags with an insecticide may be also an important adjunct in controlling insect pests in stored grains and seeds (4, 29, 65, 89, 111).

Coated plate method.—Non- or little-absorptive substrata such as glass and plywood have been used often for studies of relations between physical or morphological features of insecticide residues and their toxicities. A large variation in the toxicity of DDT residues to house flies has been reported by Woodruff & Turner (132), who deposited the residues on glass slides, to which the flies were attracted by light. After the flies were exposed for 1 to 2 hr., the treated slides were covered by bottles to prevent further exposure. As a general rule, the deposit of DDT was varied by changing the concentration of the spray suspension or solution. Turner & Woodruff (127, 128) have studied factors affecting the toxicity of DDT residues by the coated plate technique.

Lindquist *et al.* (76) exposed the boards, petri dishes and glass plates sprayed with DDT solutions, emulsions, and suspensions to ultraviolet light and sunlight and evaluated the effect on DDT residues by the time required to give a knockdown of house flies. To study the relation of droplet size to toxicity in residual-type DDT-oil spray, Anderson (2) placed the anaesthetized *Drosophila* in petri dishes together with food; after the flies had revived, he turned the dish carefully so that the treated surface became the floor. Lindsay & Haines (77) tested the resistance of house flies to residual-type insecticides by exposing them to treated petri dishes for a uniform period of time; 24- and 48-hr. records of knockdown or kill after the test appear most satisfactory for comparing insecticides. Nelson (90) transferred the house flies immobilized by chilling to a petri dish and inverted it on a clean glass plate. When all the flies had recovered from the chilling, he pulled the dish onto another glass plate, the surface of which was covered with the residue to be evaluated. With this same method, Chisholm *et al.* (26) studied the effect of sunlight on the DDT deposit.

Teotia & Dahm (124) have studied the effect of temperature, humidity, and weathering on the residual toxicities to the house fly of aldrin, chlordane, dieldrin, lindane and parathion deposited on various plates. A special technique utilizing glass, wood, and mud plaster was developed by Pal (94, 95) to test the effectiveness of residual deposits of insecticides to the house flies. This technique proved fairly sensitive in detecting difference in knockdown and mortality rates of various deposits, and the relationship between the physical forms of DDT and their effectiveness was discussed. Tsao *et al.* (126) compared the evaporation rates and toxicity to house flies of

lindane and lindane-chlorinated polyphenyl deposits. Lindane deposits lost weight rapidly, but, with the addition of an equal part of chlorinated polyphenyl, the rate of evaporation was cut down markedly. Chlorinated polyphenyl itself had no toxic effect on the house flies, but when incorporated with lindane it increased markedly the residual toxicity of that insecticide. Using adult house flies, Wingo (131) studied the effect of direct sunlight and heat on residues of Diazinon on glass plates and concluded that both heat and direct sunlight materially reduce subsequently toxicity of Diazinon residue.

Burt & Ward (16) studied the persistence and toxicity of DDT crystals on thin layers of plant wax. Residues of crystalline DDT at rates of from 2 to 8 μg. per sq. cm. were formed on glass plates, plain or coated with a 0.5 μ layer of wax obtained from leaves of sisal or cabbage. DDT remaining after storage was estimated biologically by means of its contact action on adults of *T. castaneum*. Loschiavo (80) investigated the rate of oviposition of *T. confusum* surviving the exposure to residues of *p,p'*-DDT. A plastic ring 2 cm. deep and 10 cm. in diameter was placed on the test plates to confine the insects during exposure. The rate of oviposition of *T. confusum* surviving exposure to 10- or 25-per-cent residues of *p,p'*-DDT was signficantly reduced during a period of observation from the seventh to the twenty-sixth day after exposure; this effect was not observed in females surviving exposure to 1-, 3-, or 5-per-cent residues. Females surviving exposure to 5-per-cent DDT laid significantly fewer eggs than unexposed females during the four days following exposure, the fewest being laid on the first day. In laboratory tests, Granett *et al.* (53) found that allethrin residues of 144 mg. per sq. ft. retained toxicity for several months against house flies. Pyrethrins caused knockdown of flies up to 1 month after application, but the flies recovered from the effect of pyrethrins residues when tested 1 week after application. When allethrin was combined with a synergist, the amount of residue could be reduced to 28 mg. per sq. ft. In a similar manner, the residual action of the pyrethrins was markedly improved. When residues of 144 and 72 mg. per sq. ft. were exposed to ultraviolet light, the allethrin residue was affected in about 30 hr., whereas pyrethrins were relatively unaffected.

Crowell & Fay (31) applied DDT and other halogenated hydrocarbon insecticides to glass by spraying them directly in molten form. The residues of these insecticides at 200 to 400 mg. per sq. ft. gave 97- to 100-per-cent mortality of female house flies in 24 hr. after 15 min. exposure. A residue of 200 mg. per sq. ft. of DDT showed excellent residual activity for 12 weeks in the laboratory. Metcalf & Wilson (86) used beaverboard sprayed or brushed with pyrethrins to study the residual toxicity to *Anopheles quadrimaculatus* Say and *Aedes aegypti* (Linnaeus): results warrant use of residual applications of pyrethrins for the control of adult mosquitoes in dwellings. Gahan *et al.* (50) have shown that DDT residues on wood and canvas remained toxic to *A. quadrimaculatus* for quite a long period of time. Granett *et al.* (53) brushed desired amounts of allethrin in acetone solution

on freshly sanded plywood and exposed flies to the coated panels under cellophane-covered embroidery-hoop cages. Subsequent tests were made by spreading a solution or aqueous emulsion evenly over a panel from a pipette. Using *Anopheles vagus* Dönitz, Reid (105) tested the residual effects of DDT, BHC and chlordane as wettable powders on plywood panels; the order of effectiveness was DDT, BHC, chlordane. Cutkomp (32) found that males of *A. quadrimaculatus* were more rapidly knocked down by these residual films than females.

In screening DDT-synergist combinations for the control of resistant flies, Sumerford *et al.* (115) used a formulation in which 0.5 per cent of the test chemical and 5-per-cent DDT were combined in a methyl ethyl ketone solution. Each test solution was pipetted onto poster-board test panels and adult house flies were confined in a petri dish cage on the residues. In special evaluations of vibration effects, Crowell & Fay (31) used a vibrator which consisted of a master panel actuated by a low-voltage solenoid energized by 60-cycle A. C. current. Simmons (110) described the results of exposure for various periods of *A. quadrimaculatus* to several types of wall surface treated with specified doses of DDT. Suzuki & Toyama (120) studied the residual toxicities of DDT, lindane, and lindane synergized with resin acids to the common house fly, *Musca domestica vicina* Macquardt. The residual toxicity of lindane was improved considerably by the use of resin acids. The periods of time that the toxicity of DDT persisted were in the order of gypsum plate > glass plate > wooden board, but the order in the case of lindane was wooden board ≒ gypsum plate > glass plate. There were no remarkable differences of residual toxicities between the kerosene solution and emulsion of either of the two insecticides.

Coated cage method.—In the film method described above, the test insects may walk or rest on either the treated or the untreated surface. In order to make the test insects touch the insecticide residue continuously, cages must be constructed in such a manner that all the sides, top, and bottom are constructed with treated panels. A quart jar, Mason fruit jar, or soup strainer may be used, and insecticides must be applied on the entire inner surface of them. Gahan *et al.* (50) found that DDT is effective as a residual-type spray against adults of *A. quadrimaculatus* and *A. aegypti* when applied to wooden boxes and canvas cages at the rate at least 10 mg. per sq. ft. Lindquist & McDuffie (75) employed a small, cylindrical screen-wire cage that had been treated with DDT at the rate of 200 mg. per sq. ft. to determine the speed of knockdown of *Anopheles albimanus* Wiedemann. Kartman & Silveira (69) reported that females of *Anopheles gambiae* Giles were allowed 60-, 30-, and 5-sec. contact with an aspirator tube, the inside of which is coated with residues of a dosage of 125 mg. of DDT per sq. ft., and that they showed typical DDT toxicity in less than 10 min. after contact. Furman & Hoskins (49) used shell vials and house flies to get the reference mortality curve for the detection of BHC in terms of gamma isomer equivalent. Gahan *et al.* (51) sprayed γ-BHC, DDT, chlordane, and chlorinated camphene on the inner surface of plywood boxes

to compare their efficiency as residual deposits to mosquitoes, house flies, and cockroaches. DDT was the only one causing high mortalities of *M. domestica* and *A. aegypti* even at the end of 36 weeks at all dosages tested.

Siegler (109) tested the residual toxicity of some organic insecticides to *Popillia japonica*. In the test with aged residues, open, empty cages were conveyed through the spray, and the beetles were confined in the cages several days later; unsprayed slices of apple were provided as the food. Fay *et al.* (44) devised a method for detection in the field of adult-mosquito resistance to DDT residue. The test procedure involved simply constructed, portable test equipment. Barnes *et al.* (6) exposed three strains of grape leafhopper, *Erythroneura variabilis* Beamer, to DDT in Erlenmeyer flasks. Natural mortality of the leafhoppers was effectively reduced by providing them with water by means of a piece of absorbent cotton inserted through a tube wrapped in the cellulose cotton flask stopper. The results show that the Verdemont strain was the most susceptible of the three to DDT, the Indio strain was significantly less susceptible than the Verdemont strain, and the Arizona strain was very resistant (48-hr. exposure brought no mortality).

Ishikura & Goto (62) used small vials to study the effect of temperature on the toxicity of organic phosphorus insecticides against the rice weevil, *Sitophilus oryza* (Linnaeus), and the azuki bean weevil, *Callosobruchus chinensis* (Linnaeus). The results showed that the rise of temperature increased remarkably the insecticidal properties of parathion as well as TEPP. Ishikura & Ozaki (64) studied by the same method the resistance of *C. chinensis* to BHC emulsion. The beetles emerging earlier were the most susceptible to BHC; tolerance increased markedly in individuals which emerged later, and it attained highest level in those appearing just before the peak of emergence. The BHC tolerance was positively correlated with the elytral length. The highest LD_{50} obtained was 9.3 times the lowest in the female and 10.5 times in the male. Gannon & Decker (52) confined the chinch bugs, *Blissus leucopterus* (Say), in pint ice-cream cartons treated with various insecticides. Equivalent dosage rates are indicated as follows: endrin, 1; parathion, 1; lindane, 2; dieldrin, 3; heptachlor, 16; chlordane, 32; toxaphene, 32; and DDT, 96.

To learn the DDT tolerance of adult lygus bugs (*Lygus* spp.) on seed alfalfa, Andres *et al.* (3) used vials modified from the type described by Hoskins & Messenger (60). The lygus bugs were exposed to the standardized oil-DDT mixture in the bioassay vials and then transferred to holding cages which contained moistened cotton and a piece of fresh string bean. The tolerance to DDT of lygus bugs increases during the growth of the alfalfa seed crop. At harvest time the lygus bugs in alfalfa seed fields are 3 to 4 times as tolerant of DDT as those from untreated alfalfa hay fields and as they were at the beginning of the seed season.

Using small cages and screens, Linsley & MacSwain (78) and Linsley *et al.* (79) compared the susceptibility of wild bees and honey bees to DDT. It has been found that neither reacted under such conditions of confinement

in the manner that would be expected in more natural circumstances.

Cage method.—For the evaluation of the effectiveness of insecticide residues deposited on the surface of fruits, foliages, and other plant materials, the so-called laboratory cage method has been resorted to very often. Sometimes, treated branches bearing fruits or leaves or other plant materials are placed in screen cages, quart or pint Mason jar with cheesecloth covers, or petri dishes, etc. Often the stems are placed in water to prevent wilting, and also wet filter paper or small pieces of cotton wool are placed in the jars or petri dishes to keep them moist. Then a suitable number of test insects are exposed to the insecticide residues.

With modified Munger cells constructed of plastic, Bartlett (7) studied the retentive toxicity of weathered insecticide residues on aged sprayed leaf surfaces to entomophagus insects associated with citrus pests. He used a suction tube for preventing vapor accumulation and for maintaining constant temperature and humidity within the cells. The materials most deleterious to the beneficial insects tested were DDT, parathion, lime-sulfur, TDE, EPN, and sulfur, named in decreasing order of toxicity. Aramite and ovex (*para*-chlorophenyl-*para*-chlorobenzene sulphonate) were practically nontoxic.

Johansen *et al.* (66) tested the residual toxicity of methoxychlor and parathion deposited on cherry leaves to the cherry fruit fly, *Rhagoletis cingulata* (Loew). In laboratory tests, the LD_{95} of methoxychlor for the cherry fruit fly was about 33 times that of parathion for the first 24 hr. However, the commonly employed field dose of methoxychlor for the cherry fruit fly is only 4 times that of parathion. Hornstein *et al.* (59) tested the persistence of lindane-chlorinated terphenyl residues on outdoor foliage. Grass samples were cut at the base of the blades and were placed in pint Mason jars with house flies. The results show that the lindane-chlorinated terphenyl residues on outdoor foliage give longer protection than lindane alone. Sullivan *et al.* (114) determined the effectiveness of residues on outdoor foliage of both nonvolatile and volatile chlorinated organic insecticides, alone and in combination with chlorinated terphenyl. He sprayed methyl ethyl ketone solutions of the insecticide mixtures on pine branches outdoors and periodically collected twigs for indoor tests on house flies. It was found that a chlorinated terphenyl greatly extended the residual life of lindane and aldrin, but that DDT was not helped. Surface residues from methyl ethyl ketone solutions of DDT lasted longer, however, than fuel-oil applications.

Ward & Burt (129) compared the rates of loss of DDT deposits from glass plates and growing leaves: leaf discs were cut from the sprayed leaves, and adults of *T. castaneum* were confined on their surfaces by means of small glass collars.

Experiments and observations were made by Allen (1) to determine whether parasites of the oriental fruit moth, *Grapholitha molesta* (Busck), are more susceptible than their host to residual insecticides such as DDT and parathion. When DDT of the usual concentration was sprayed on paper toweling and allowed to weather, the deposits lost much of their toxicity to

the oriental fruit moths in less than 3 weeks, but remained highly toxic to adults of *Macrocentrus ancylivorus* Rhower for 5 weeks. Similar results were obtained when adults of *G. molesta* and *M. ancylivorus* were introduced in cages placed over small peach trees sprayed with DDT. In laboratory tests with immature apples from sprayed orchards, DDT residues were highly toxic to *M. ancylivorus* and *Horogenes molestae* (Uchida), but only slightly so to *Phanerotoma grapholithae* Muesebeck, *Agathis festiva* Muesebeck, and *G. molesta*. Light parathion residues were extremely toxic to *H. molesta, P. grapholithae,* and *G. molesta,* but not to *A. festiva.* Very small residues of parathion not toxic to either *P. grapholithae* or *A. festiva* appear to have caused a delayed mortality of *G. molesta* (first noticeable after 96-hr. exposure).

Residues in soil.—Fleming (46) studied the persistence of DDT in 28 types of soil when applied at the rate of 25 lb. per acre to control larvae of the Japanese beetle. Schwedtfeger (108) observed the mortality of various instars of *Melolontha hippocastani* Linnaeus when the insects were placed upon the surface of soils treated with γ-BHC. Fleming *et al.* (47) described a method of biological assay of soil residues of chlordane and DDT, alone and in combination, using the oriental-fruit-moth parasite *M. ancylivorus* as a test insect. Extracts of soils made with a mixture of benzene and isopropanol were clarified with activated charcoal. When only one toxicant was present, the extract was placed on filter paper in petri dishes and the reaction of the insects to the residue of each test sample was compared with the reactions to known residues of the toxicant. When both materials were present, the chlordane was assayed first and then removed by wetting and drying the filter papers before assaying the DDT residues. Possibly the most practical procedure would be to determine the total organic chloride chemically and the chlordane biologically and to calculate the DDT from the organic chloride in excess of that in the chlordane. The biological assays indicated that the chemical determination of total organic chloride in the soil is a good basis for estimating insecticidally effective amounts of either chlordane or DDT. Preliminary tests showed that other chlorinated hydrocarbons in soil may be determined biologically.

Kloke (72) determined the residual effectiveness of insecticides by placing *Acanthoscelides obtectus* (Say) in contact with the soil 18 months after treatment. BHC-treated soils gave 60 to 100 per cent kill; DDT, 45 to 50 per cent; and parathion, 30 to 35 per cent. Wylie (133) determined insecticide residues in soil by exposing adults of *D. melanogaster* to soil moistened with Karo solution plus a graded series of dilutions of insecticides. To prepare the soil samples, the lumps were broken up and screened to remove small stones. Five gm. of air-dried soil were then weighed into each of four vials and moistened with 2 ml. of an aqueous solution containing 25 per cent by volume of white Karo. A glass stirring rod was used to mix the contents and to spread it on the side of vials to a height of approximately 2½ inches. This method, however, is considered to be inadequate for lead arsenate and methoxychlor.

Residues in Plant and Animal Materials

For assaying residues in plant and animal materials, some preliminary work is required to produce an extract free from interfering toxic substances. Nolan & Wilcoxon (92) obtained parathion from onions, potato foliage, corn foliage, and beans by first macerating the plant material with an equal weight of water and then extracting the resulting suspension with benzene. Various amounts of this solution were pipetted into beakers, and when the benzene was evaporated with an air jet the residue was dissolved by adding acetone. Newman (91) reported that a procedure almost similar to that mentioned above was satisfactory for the estimation of parathion. Heating was avoided at all stages of the procedure. Kocher *et al.* (73) recommended grinding of vegetable material with anhydrous sodium sulphate, the water being fixed as water of crystallization. Thus an anhydrous extract was prepared by boiling with ether under reflux; then, after the ether had been distilled off, the residue was taken up in a little ethanol. For γ-BHC, chromatographic methods of purification were described by Hoskins *et al.* (61), who ran petroleum-ether solution through silica columns and then eluded with petroleum ether and 5-per-cent benzene. Laug (74) extracted DDT from animal tissue and excreta with ether. Wet or dehydrated tissues were pulped with a small quantity of washed sea sand in a mortar. Ten ml. of ether were successively added, thoroughly mixed with the tissue brei, and decanted into a volumetric flask of an appropriate size. In the case of fluids such as blood, urine, or bile, successive ether extractions were made in the separatory funnels. Emulsions can usually be broken down with a few drops of absolute alcohol.

Use of methods for residues on substrata.—Laug (74) evaporated an aliquot of the ether solution containing 2.5 to 10 μg. of DDT in the test flask. The residue after evaporation of the ether will contain, besides DDT, varying amounts of fat or other ether-soluble extractives. It has been found that ether-extract residues from more than 0.5 to 10 gm. of tissues, particularly liver and kidney, make the DDT assay unreliable. Fortunately, however, the toxic effects of tissue extractives on the fly can be sharply differentiated from those of DDT. By means of this expedient, it has frequently been possible to guess roughly amounts of DDT smaller than 2.5 p.p.m. in tissues; this is the lower limit of determinable DDT. The flies were exposed to DDT in widemouthed Erlenmeyer flask. Moisture in residues, which results from condensation of water, must also be completely removed. After the test vessels with DDT residues are ready, they are weighed to the nearest 0.01 gm. and are then ready to receive the flies. After 100 flies are admitted, the test flask, is quickly capped with a perforated filter paper and again weighed. The flies are allowed to remain in the flask for about 20 hr. (Some biological and physical conditions affecting the dosage-mortality line were also discussed in Laug's paper.) Carter *et al.* (25) evaluated the residues of toxaphene, BHC, and DDT contained in beef fat by modifying Laug's method. To separate the toxicant residue, portions of fat were treated by the Schechter-Haller method. The residues

were placed in 500 ml. narrow-mouthed beaker flasks, which were then turned on their sides to receive approximately 100 flies each. As soon as the flies, which had been immobolized by chilling, revived, the flasks were set upright and observations made on knockdown and kill. Skim milk was provided on strips of cotton hung from the mouths of the flasks, which were covered with cheese cloth. Dahm & Pankaskie (33) described a method for determining aldrin which consists of exposing flies to residues of the test material in widemouthed quart jars.

Stansbury & Dahm (112) determined the effect of commercial-alfalfa dehydration upon residues of four insecticides: aldrin, chlordane, parathion, and toxaphene. The results of over 400 assays showed that residues of the four insecticides were reduced considerably by the dehydration process. Carter *et al.* (25) evaluated the toxaphene residues on alfalfa hay by Campbell's turntable method (67). Hoskins *et al.* (61) found that the addition to each jar of a suitable amount of light oil, which forms an almost invisible film, after evaporation of the solvent, ensured a uniformity of pickup of insecticides. With an exposure time of about 30 min., a 50-per-cent kill of flies can be produced by 0.9 μg. of γ-BHC per jar—a result which corresponds to a 78-per-cent pickup of insecticide. In this test TEPP appeared somewhat less toxic, the amount necessary for a corresponding mortality being 6 to 7 μg. per jar. Klein *et al.* (71) assayed endrin biologically in leafy vegetables by a procedure consisting of the following steps: preparation of samples by grinding, extraction of the insecticides with petroleum ether, removal of organic impurities from the extract by chromatography, extraction of the insecticide with acetonitrile, refluxing with KOH-alcohol reagent, and evaluation of endrin content by means of the bioassay according to Laug's method. Endrin at the 0.1 and 0.5 p.p.m. levels was evaluated with an accuracy of approximately 70 and 85 per cent. The accuracy of the assay with the fly averaged ± 0.027 p.p.m. in the extracts containing 0.13 to 1.0 p.p.m. of endrin. When 0.10 p.p.m. or less was present, the average deviation was ± 0.014 p.p.m.

Mosquito-larvae method.—Mosquito larvae are usually extremely sensitive to various insecticides, are easily cultured or can be readily collected from field, and hence are used very widely for the biological assay of minute insecticide residues. Using the larvae of *A. aegypti*, Nolan & Wilcoxon (92) have perfected a method of bioassay for parathion in the plant material. The residue extracted is dissolved by adding 0.5 cc. of acetone and 24.5 cc. of water; 25 cc. of water containing 10 three-day-old larvae are then added, and the beakers are allowed to stand until the number of dead larvae are counted. For preparation of the parathion standard, a benzene extract of untreated plant tissue is prepared as for the treated sample. To 24 cc. of this extract is added 1 cc. of a 0.00125-per-cent solution of parathion in benzene to obtain the standard parathion solution containing 0.05 μg. of parathion per 1 cc. of benzene extract or 0.0521 μg. of parathion per 1 gm. of plant material. This method is sensitive enough to detect about 0.01 p.p.m. parathion in plant material. Nonetheless, parathion in some plant

tissues, such as shelled peas and potato tubers, is difficult to assay because of the toxicity of the benzene extracts of untreated material. And assay is almost impossible for the material which may have been treated with more than one insecticide, and whose history is unknown. Terriere & Ingalsbe (125) showed that treatment of the benzene extracts of the potato tissue with 0.2-per-cent sodium hydroxide would remove toxic substances in the extracts without apparent effect on any insecticide residues present. The lowest concentration of insecticides which could be detected in the potato tissue were as follows: for aldrin, dieldrin, BHC, DDT, and heptachlor, 0.02 p.p.m.; for toxaphene, 0.04 p.p.m.; for chlordane, 0.06 p.p.m.; and for parathion and EPN, 0.005 p.p.m. Terriere & Ingalsbe studied further the problem of the translocation of soil insecticide residues into crops.

By a modified version of Nolan & Wilcoxon's method, Hartzell & Storrs (56) also assayed the known amounts of spray residues of ten insecticides [viz., heptachlor, aldrin, dieldrin, chlordane, lindane, 2-nitro-1, 1-*bis*(*p*-chlorophenyl)propane, 2-nitro-1,1-*bis*(*p*-chlorophenyl)butane, methoxychlor, TDE, and toxaphene] in processed food such as green beans, and mixtures of apricots and apples. The above-mentioned insecticides could be readily detected when added to the processed foods at concentrations of 0.5 to 5.0 p.p.m. Benzene extracts of such samples were diluted so as to contain 0.01 to 1.0 p.p.m. of the insecticides for critical assaying. The critical dilution of extracts of aqueous suspensions, below which bioassay was impossible, was 0.125 to 0.01 p.p.m. Utilizing the third instar of *A. aegypti,* Newman (91) detected a concentration of 0.02 p.p.m. of parathion in a 100-g. sample of contaminated material. With the same method, he assayed parathion residues in soil, in tomatoes and fruit and in toxic smoke residues, and he also determined the relative toxicities of phosphorus insecticides. Bushland & King (17) have discovered that mosquito larvae tolerate up to 1 per cent of acetone in the test medium.

When chlorinated hydrocarbon insecticides contaminate milk, they are found only in the butterfat. Bushland (20) obtained butterfat by churning cream, melting the butter, and decanting the fat, then emulsifying by mixing one part of sorbitan trioleate and one part of polyoxyethylene sorbitan trioleate with 8 parts of melted butterfat. For the mosquito larvae test, he emulsified the mixture in warm distilled water by means of a homogenizer. Tests with the fourth instar larvae of *A. aegypti* showed that most of the larvae could survive for 24 hr. in 4-per-cent butterfat emulsified as just described. Butterfat samples containing 25 p.p.m of the various insecticides were prepared by dissolving weighed amounts of the technical material in hot butterfat. The samples were emulsified and tested for toxicity. Bushland also made the tests with acid-treated butterfat. Four-gm. samples of butterfat containing 25 p.p.m. of insecticide were dissolved in chloroform and treated with a sulfuric acid–fuming sulfuric acid mixture. After evaporation of the chloroform, the flasks were washed with 4 ml. of acetone. The acetone washings were diluted 1: 100 with distilled water to prepare samples

containing 0.25 p.p.m. of the insecticides. The preparations at this and further dilutions were tested with 25 larvae of *A. aegypti* in 100 ml. of water at 80°F. The results suggest that this biological method was sufficiently sensitive to detect 0.5 p.p.m. of lindane in milk of 4-per-cent butterfat content. In testing with the beef fat, chlordane was dissolved in melted beef tallow so as to give concentrations ranging from 1 to 8 p.p.m., and 25 gm. samples were dissolved in chloroform and treated with a sulfuric acid–fuming sulfuric acid mixture. The chloroform solutions were concentrated and transferred to test tubes, and the remaining chloroform was removed by evaporation. A measured amount of acetone was added, and the test was carried out with *Anopheles quadrimaculatus* Say.

Oral-administration method.—Taking advantage of the house fly's natural habit of ingesting a large variety of food products, oral-administration methods were devised for the biological assay of insecticide residues. It is now a well-established fact that lactating animals on a diet containing DDT may secrete this substance in their milk. Carter *et al.* (24) reported, however, that DDT in synthetic creams at concentrations of 50 p.p.m. or less could not be definitely determined on the basis of fly mortalities. Sun & Sun (116) devised a method of determining insecticides in milk by feeding house flies homogenized milk directly. The tests were carried out on test milks in widemouthed pint jars, with milk soaked on cotton serving as control. In this way it is possible to detect such low concentrations as 0.1 p.p.m. of dieldrin or aldrin, 0.2 p.p.m. of lindane, 0.5 to 0.6 p.p.m. of isodrin or endrin, and 1.4 p.p.m. of DDT.

Frawley *et al.* (48) made an ether extract of the sample, evaporated it to dryness over sugar, dissolved the sugar in an aqueous medium, and allowed the house flies to feed *ad libitum* on the solution for 24 hr. This method was applied to several types of food products, which included apples, cucumbers, strawberries, peas, and yeast, and was sensitive enough to detect 3 μg. of parathion, 4 μg. of EPN, 5 μg. of lindane, and 15 μg. of DDT. Fischer & Smallman (45) prepared water solutions from a 0.05-per-cent acetone solution of aldrin, macerated fresh or canned custard pumpkin, and the toxicant, and spread it as the food evenly along a strip of filter paper in a test tube. When a number of *D. melanogaster* had been introduced, the tube was placed in a horizontal position so that the filter paper formed a roof. Moisture condensed on the glass, and the weakened, badly affected flies became trapped on the wet glass. All flies dead or trapped were recorded as dead. The results show that valid estimates of aldrin may be made at levels of 0.05 p.p.m. To test the accuracy and sensitivity of the method, a standard was prepared by adding a water suspension of aldrin to pumpkin which was then serially diluted with further known weights of pumpkin. This standard was then compared to a simulated "unknown" prepared in exactly the same way, except that a small "residue" of aldrin was added initially. The serial dilution of the "residue" as well as the standard provides a regression line parallel to the standard, and so comparisons

can be made at any level on the line. This method differs from that of Sun & Sun (117), wherein one dosage is added to the residue-containing tissue and the resulting mortality plotted on a calibration curve.

Sun & Pankaskie (118) devised a method which consists of exposing the adult *D. melanogaster* to macerated plant tissues. The concentration of the insecticide-acetone solution added to the macerated, untreated tissue for making the standard should be such that not more than 0.5 ml. of acetone solution is added to 2.5 gm. of sample. The same amount of acetone was added to treated samples to compensate for any possible effect on the flies. About 20 gm. of the macerated tissue were placed into each of six 4-oz. widemouthed glass jars. Fifty 1-day-old adult flies anaesthetized by carbon dioxide were put into a small glass cup which in turn was placed in the jar with the plant tissue. Mortality counts were made and recorded 18 to 48 hr. later, when the mortality in the standards range from 30 to 80 per cent. The amount of insecticide in the treated sample was determined by comparing the percentage mortality obtained from exposure to the treated plant tissues with the standard reference curve plotted. As little as 0.1 p.p.m. of aldrin or dieldrin could be detected in many fresh and canned fruits and vegetables, such as potatoes, peas, spinach, etc.

When pomace flies are used for the bioassay of dry residues deposited from the evaporation of plant or animal tissue extracts, the methods described by Sun & Sun (117) for house flies can be employed. Minor differences are that the extract is deposited on the bottom of a 4-oz. widemouthed jar, that pumpkin serves as the food for pomace flies, and that each jar is covered with a piece of tissue paper. This method is satisfactory for determining the amount of aldrin and dieldrin in the extracts of brain, liver, kidney, muscle, and fat obtained from rats used in toxicological studies. As little as 0.05 μg. of aldrin or dieldrin per test jar can be detected by this method. Eddy *et al.* (42) undertook a study of the effects on some insects [viz., *Siphona irritans* (Linnaeus), *A. aegypti, Stomoxys calcitrans* (Linnaeus), *Callitroga macellaria* (Fabricus), and *Callitroga hominivorax* (Coquerel)] feeding on the blood or on the manure of Hereford cattle fed for different periods on a diet containing various chlorinated hydrocarbon insecticides.

The classical cage method for evaluating the effectiveness of stomach poisons may be included in the category of direct-feeding, and also the poison baits used for the control of locusts, flies, cockroaches, crickets, etc. could be placed in the same category.

Photomigratioin method.—Recently, Burchfield *et al.* (11, 15) have devised an unique method for the bioassay of insecticide residues based on the observation that mosquito larvae move rapidly away from an intense light and congregate where the net illumination is least. This response is reduced by the presence of toxic materials. One hundred second instar larvae of *A. aegypti* 20 to 22 hr. old, are confined behind a porous barrier in a semicircular glass trough 10 in. in length. The trough contains 1 ml. of a solu-

tion of the insecticide or plant extract in acetone or carbitol and 99 ml. of water. When a 500-watt light source parallel to the longitudinal axis of the trough is turned on and the barrier removed, viable larvae rapidly migrate to the far end. After an exposure of 1 min., a second barrier is dropped into place and the larvae trapped behind it are counted and classified by an arbitrary physiological response which is independent of any personal judgment on the part of a laboratory technician. The time is recorded and the experiment repeated at intervals until 70 to 90 per cent of the larvae have become inactivated. Percentages in probits are plotted against time, and the time required for inhibition of 50 per cent of the population (T_{50}) is estimated by interpolation. The T_{50} is proportional to the concentration of insecticide; hence the amount of toxic material in an unknown sample can be estimated by comparison with T_{50} values obtained at known concentrations of the same substance. This relationship is not linear, but it is usually satisfactory for interpolating results from a graph of the logarithm of the T_{50} against the reciprocal of the concentration in p.p.m. Kinetic studies on this relation have been carried out in detail by Burchfield & Storrs (14). Burchfield *et al.* (12) have also suggested a number of improvements for rearing larvae of *A. aegypti* for use in insecticide bioassay by the photomigration test and have pointed out various precautions that must be taken during rearing and testing.

As shown in Table I, most insecticides used in agriculture inhibit the photomigration of 50 per cent of the larvae in 1 hr. or less at concentrations of 0.1 to 1.0 p.p.m. Many are highly effective at 0.01 p.p.m., and, in the case of a few insecticides such as lindane, heptachlor and aldrin, T_{50} values can be obtained at 0.002 p.p.m. in less than 5 hr. When required, smaller amounts of toxicants can be detected with larger samples or longer reaction periods. This high sensitivity and the broad range of activity measurable at a single concentration are advantageous particularly when the nature and amount of the residue are unknown. Mixtures of insecticides represent a special case which cannot be evaluated without some knowledge of the components (113). In all cases so far observed, they would not result in a serious masking of toxicity.

Not all physiologically active materials can be effectively utilized for the T_{50} test. The evidence to date indicates that the test is more or less specific for liposoluble compounds which act as neural or muscular poisons. The fact that many general metabolic poisons do not produce a rapid response in the photomigration test greatly reduces the possibility that observed toxicity may be caused by naturally occurring plant products, with some obvious exceptions such as the pyrethrins. Organic acids and alkaloids do not produce an effect except at high concentrations. However, as in most bioassay methods the presence of lipides tends to mask toxicity through preferential solubilization of the insecticide in the oil globules (57). Thus in the presence of 100 p.p.m. olive oil, the T_{50} value obtained with a solution containing 0.1 p.p.m. heptachlor is increased from ca. 30 min. to ca. 100 min.

TABLE I

TYPICAL T_{50} VALUES OBTAINED AT FOUR CONCENTRATIONS OF VARIOUS INSECTICIDES

Insecticide	T_{50} in minutes at concentration (p.p.m.)				Optimum bioassay range (p.p.m.)
	10	1	0.1	0.01	
Allethrin	1	2	43	300	1.0 to 0.1
Lindane	3	7.5	25.4	182	0.2 to 0.01
Methoxychlor	4	4	31.4	300	0.2 to 0.08
Parathion	7.8	19.0	50	300	0.2 to 0.02
DDT	8.3	11.6	18.4	116	0.1 to 0.005
Heptachlor	15.5	16.0	29.5	93.5	0.1 to 0.005
Chlordane	16.7	22.1	47.2	170	0.2 to 0.01
Aldrin	26.6	31.3	45.4	148	0.2 to 0.005
Toxaphene	36.5	46.2	144	300	1.0 to 0.05

The plant pigments such as the chlorophylls, carotinoids, and xanthophylls can be effectively removed by chromatographic adsorption on calcium carbonate (11) or on florisil (57), but the nonadsorable glycerides and waxes which remain still constitute a serious interference. Where sensitivity can be sacrificed, this defect can be compensated for by making the reference standards with a constant amount of glyceride corresponding to the weight of the extract. Partition of the insecticide and lipide between acetonitrille and hexane (68) or between dimethylformamide and hexan (13) is potentially useful. For the photomigration method, steam distillation is particularly attractive when the insecticide is volatile enough (57). Lindane and heptachlor can be recovered almost quantitatively from olive oil by this method, but the recoveries of DDT and methoxychlor are less satisfactory.

The photomigration method has been used for the estimation of DDT, methoxychlor, lindane, parathion, aldrin, heptachlor, and chlordane, in extracts from raw and processed food crops such as potatoes, yams, cabbage, turnips, onions, corn, and carrots. Extensive comparisons were made of the chemical (34, 100) and bioassay (11) methods for the determinaton of traces of chlordane and heptachlor in food crops. The average chlordane content obtained on 64 samples representing the seven different crops was 0.035 p.p.m. by chemical analysis as compared to 0.033 p.p.m. by bioassay. The best agreement was obtained with carrots, where the ratio of chemical analysis to bioassay was 1.0, and the poorest with yams, where the ratio was 1.9. Correlation coefficients varied from -0.185 to $+0.695$ with a weighted average of $+0.175$. The best results were obtained on extracts of onions where the ratio of chemical analysis to bioassay was 1.3 and the poorest on yams where the ratio was 8.7. The average heptachlor content of 80 samples based on the fresh weight of the crop was 0.017 p.p.m. by chemical analysis and

0.007 p.p.m. by bioassay. Correlation coefficients for each crop varied from +0.906 to −0.053 with an average weighted value of +0.252. Over-all correlation was significant at the 5-per-cent level with an average coefficient of +0.219 and 115 degrees of freedom. This low correlation coefficient resulted from the facts that the methods were used close to their limits of sensitivity and variations in insecticide content between samples were small. The colorimetric methods tend to give high readings since impurities present in the extracts cause background absorption. On the other hand, bioassay results are generally low since lipides reduce the rate of insecticidal action. The true values are probably somewhere intermediate between the two extremes.

Bioassay with Fish and Crustacea

Bioassay with small fishes has been extensively employed as a tracer technique in the studies of many insecticidal materials of botanical origin and also applied for the determination of insecticidal residues, since various synthetic compounds, such as dieldrin and aldrin, are extraordinarily toxic to fish. The goldfish, *Carassius auratus* Linneaus, has been most extensively used for this purpose, as it is readily available, inexpensive, and easily handled and cared for. We owe much to Gersdorff for the development of the method with goldfish. Results of experiments carried out with his method have been reported by many authors. The common top minnow, *Gambusia affinis* (Baird and Girard), also has been used by Carpenter, Gunther, Odum & Sumerford, Yardin, and it is recommended as a test organism having many advantages over the goldfish; for example, they are very hardy and are less affected by moderate temperature changes. Guppies (*Lebistes reticulatus* Peters—a species of Poeciliidae) were used by Pagan and Pagan & Morris. Fortunately, a review of these contributions has been fully made by Gunther & Blinn (54). Formerly, Cobitidae species such as *Misgurnus anguillicandatus* (Cantor) were used as the test organisms for rotenoid by Takei *et al.* (121) and Takei & Miyajima (122).

Recently biological screening test utilizing the goldfish for chlorinated insecticides has been developed by Davidow & Sabatino (35). It consists in introducing two 2-inch goldfish to each of a series of solutions containing increasing quantities of the insecticides. Solution in water was prepared by first dissolving the insecticide in acetone and then adding 0.1 ml. of the acetone solution to 250 ml. of water. The concentration of 0.04-per-cent acetone has no adverse effect upon the goldfish. The symptoms of a strong poisoning are, first, hyperexcitabilty, then convulsions and loss of equilibrium; death ensues within 3 hr. To lower doses the fish may not succumb, even though they exhibit the other symptoms of poisoning. Convulsions and loss of equilibrium were chosen as the most suitable indicators of poisoning. By this method, the approximate limits of sensitivity of goldfish to DDT, lindane, heptachlor, toxaphene, dieldrin, chlordane, methoxychlor, Dilan, and aldrin have been established. It was found that microquantities of these chlorinated insecticides should be steam distilled, and approximately all of the quantity

of insecticide was present in the first 200 ml. of distillate. Furthermore, it was found that the distillate from 100-gm. samples of carrots, apples, lima beans, grapefruit and orange juice, milk, and alfalfa had no toxic effect upon the fish during the 3-hr. observation time. Positive results were obtained with food containing 7 p.p.m. of DDT, 5 p.p.m. of lindane, 7 p.p.m. of toxaphene, 14 p.p.m. of methoxychlor, and 7 p.p.m. of Dilan. In the case of heptachlor, chlordane, aldrin, and dieldrin, the fish failed to respond positively to levels of 0.1 p.p.m. Davidow & Schwartzman (36) made a study of the response of the goldfish to microquantities of endrin, isodrin, Strobane, and TDE. This method was applied for the assay of endrin in leafy vegetables by Klein *et al.* (71).

Because under certain conditions crustaceans, *Daphnia* spp., produce offspring parthenogenetically with consequent genetic uniformity, some investigators have used them for quantitative investigations. Thus, Wasserburger (130) used *Daphina magna* Straus for the quantitative detection of traces of DDT, BHC, and some other insecticides. Kocher *et al.* (73) stated that with *Daphnia pulex* (DeGeer) quantities of insecticide less than 1 mg. per kg. of vegetable could be determined with greater precision than was possible, for instance, by tests with house flies. The 100 gm. of treated plant material is ground with 200 to 500 gm. of anhydrous sodium sulfate. The mixture is extracted for 12 hr. with ether in 450-ml. flask. The extract, plus 5 ml. of ethyl alcohol, is distilled and taken up in 20 ml. of ethyl alcohol. Controls are run with extracts from plants to which known quantities of the same insecticides have been added. For assay, 0.1 ml. of an alcoholic solution of the toxicant is diluted with 100 ml. of water, and about 20 active individuals of *D. pulex* are put into the mixture. The percentage of animals unable to swim is used as a measure of insecticidal activity. The method has been applied successfully to the determination of residues in plants and has been reported to be sensitive for Diazinon in cauliflower at about the 0.7 p.p.m. level, for Isolan in peaches at about the 1 p.p.m. level, for demeton in cauliflower at about the 0.1 p.p.m. level, for parathion in cherries at about the 0.1 p.p.m. level, and for chlordane, dieldrin, aldrin, BHC, and DDT at higher levels of dissolved quantities. To overcome the difficulties presented by the lack of sensitivity of insects to insecticides of low toxicities, the use of *D. magna* has been explored by Davidow & Sabatino (35), whose preliminary studies indicated that it may be a suitable test organism. The use of the fresh-water shrimp, *Gammarus pulex* DeGeer, for bioassay of the toxicity of anticholinesterase compounds was recommended by Callaway *et al.* (23) for various reasons. They suggested the use of 3 or more batches of about 40 gammarids, each for a different dilution of the toxicant in a large petri dish held at 21.5°C.; mortality observations are to be made at logarithmically spaced time intervals. The 50-per-cent response time for each concentration is plotted on the graph against logarithm concentration to get a straight line. Although insecticide bioassay with these crustaceans is simple and rapid, it is generally not so sensitive as the bioassay with insects. Thus, Newman

(91) reported that the approximate quantity of parathion in a sample that produced 50-per-cent response was as follows: with *D. pulex*, 5 μg.; *G. pulex*, 5 μg.; *M. domestica* (oral), 3 μg.; *A. aegypti*, third instar, 0.5 μg.; and *A. aegypti*, first instar, 0.002 μg.

LITERATURE CITED

1. Allen, H. W., *J. Econ. Entomol.*, **50**, 49–51 (1957)
2. Anderson, R. F., *J. Econ. Entomol.*, **41**, 974–76 (1948)
3. Andres, L. A., Burton, V. E., Smith, R. F., and Swift, J. E., *J. Econ. Entomol.*, **48**, 503–13 (1955)
4. Atkins, W. G., and Greer, E. N., *J. Sci. Food Agr.*, **4**, 155–60 (1953)
5. Baker, W. C., Scudder, H. I., and Guy, E. I., *Public Health Repts. (U.S.)*, **62**, 597–612 (1947)
6. Barnes, M. M., Flock, R. A., and Garmus, R. D., *J. Econ. Entomol.*, **47**, 238–42 (1954)
7. Bartlett, B. R., *J. Entomol.*, **46**, 565–69 (1953)
8. Beran, F., *Pflanzenschutz Ber.*, **11**, 151–60 (1953)
9. Beran, F., and Neururer, J., *Pflanzenschutz Ber.*, **15**, 97–147 (1955)
10. Bocking, V., *Z. Induktive Abstammungs- u. Vererbungslehre*, **86**, 185–209 (1954)
11. Burchfield, H. P., Hilchey, J. D., and Storrs, E. E., *Contribs. Boyce Thompson Inst.*, **17**, 57–86 (1952)
12. Burchfield, H. P., Redder, A. M., Storrs, E. E., and Hilchey, J. D., *Contribs. Boyce Thompson Inst.*, **17**, 317–31 (1953)
13. Burchfield, H. P., and Storrs, E. E., *Contribs. Boyce Thompson Inst.*, **17**, 333–34 (1953)
14. Burchfield, H. P., and Storrs, E. E., *Contribs. Boyce Thompson Inst.*, **17**, 439–52 (1954)
15. Burchfield, H. P., and Hartzell, A., *J. Econ. Entomol.*, **48**, 210–14 (1955), also in *Boyce Thompson Inst. Plant Research, Professional Papers*, **2**, 112–23 (1955)
16. Burt, P. E., and Ward, J., *Bull. Entomol. Research*, **46**, 39–56 (1955)
17. Bushland, R. C., and King, W. V., *U.S. Dept. Agr., Bur. Entomol. Plant Quarantine, E-585*, 15 pp. (1943)
18. Bushland, R. C., McAlister, L. C., Jr., Eddy, G. W., Jones, H. A., and Knipling, E. F., *J. Parasitol.*, **30**, 377–87 (1944)
19. Bushland, R. C., McAlister, L. C., Jr., Jones, H. A., and Culpepper, G. H., *J. Econ. Entomol.*, **38**, 210-16 (1945)
20. Bushland, R. C., *J. Econ. Entomol.*, **44**, 421–23 (1951)

20a. Busvine, J. R., *A Critical Review of the Techniques for Testing Insecticides* (Commonwealth Institute of Entomology, London, England, 208 pp., 1957)

21. Busvine, J. R., and Barnes, S., *Bull. Entomol. Research*, **38**, 81–90 (1947)
22. Busvine, J. R., and Nash, R., *Bull. Entomol. Research*, **44**, 371–76 (1953)
23. Callaway, S., Kirnhuber, P., and Wilson, K. M., *Nature*, **170**, 843–44 (1952)
24. Carter, R. H., Hubanks, P. E., Mann, H. D., Smith F. F., Piquett, P. G., Shaw, J. C., and Ditman, L. P., *J. Econ. Entomol.*, **42**, 119–22 (1949)
25. Carter, R. H., Nelson, R. H., and Gersdorf, W. A., *Advances in Chem. Ser.*, No. 1, 271–73 (1950)
26. Chisholm, R. D., Nelson, R. H., and Fleck, E. E., *J. Econ. Entomol.*, **42**, 154–55 (1949)

27. Collins, D. L., and Nardy, R. V., *J. Econ. Entomol.*, **43**, 861–63 (1950)
28. Collins, W. E., and King, H. L., *J. Econ. Entomol.*, **46**, 51–53 (1953)
29. Cotton, R. T., Frankenfeld, J. C., and Strickland, W. B., *U.S. Dept. Agr., Bur. Entomol. Plant Quarantine, E-783*, 4 pp. (1949)
30. Crow, J. F., *J. Econ. Entomol.*, **47**, 393–98 (1954)
31. Crowell, R. L., and Fay, R. W., *J. Natl. Malaria Soc.*, **10**, 8–16 (1951)
32. Cutkomp, L. K., *J. Econ. Entomol.*, **40**, 328–33 (1947)
33. Dahm, P. A., and Pankaskie, J. E., *J. Econ. Entomol.*, **42**, 987–88 (1949)
34. Davidow, B., *J. Assoc. Offic. Agr. Chemists*, **33**, 886–94 (1950)
35. Davidow, B., and Sabatino, F. J., *J. Assoc. Offic. Agr. Chemists*, **37**, 902–5 (1954)
36. Davidow, B., and Schwartzman, G., *J. Assoc. Offic. Agr. Chemists*, **38**, 533 (1955)
37. Davidow, B., and Laug, E. P., *J. Econ. Entomol.*, **48**, 659–61 (1955)
38. Dean, R. W., *J. Econ. Entomol.*, **44**, 147–53 (1951)
39. Doner, M. W., *Soap Sanit. Chemicals*, **23**, 139, 141, 143, 193 (1947)
40. Eddy, G. W., and Carson, N. B., *J. Econ. Entomol.*, **39**, 763–67 (1946)
41. Eddy, G. W., and Bushland, R. C., *J. Econ. Entomol.*, **41**, 369–73 (1948)
42. Eddy, G. W., McGregor, W. S., Hopkins, D. E., and Dreiss, J. M., *J. Econ. Entomol.*, **47**, 35–38 (1954)
43. Fay, R. W., Buckner, A. J., and Simmons, S. W., *Am. J. Trop. Med.*, **28**, 877–87 (1948)
44. Fay, R. W., Kilpatrik, J. W., Crowell, R. L., and Quarterman, K. D., *Bull. World Health Organization*, **9**, 345–51 (1953)
45. Fisher, R. W., and Smallman, B. N., *Can. Entomologist*, **86**, 562–69 (1954)
46. Fleming, W. E., *J. Econ. Entomol.*, **43**, 87–89 (1950)
47. Fleming, W. E., Coles, L. W., and Maines, W. M., *J. Econ. Entomol.*, **44**, 310–15 (1951)
48. Frawley, J. P., Laug, E. P., and Fitzhugh, O. G., *J. Assoc. Offic. Agr. Chemists*, **35**, 741–45 (1952)
49. Furman, D. P., and Hoskins, W. M., *J. Econ. Entomol.*, **41**, 106–7 (1948)
50. Gahan, J. B., Travis, B. V., and Lindquist, A. W., *J. Econ. Entomol.*, **38**, 236–40 (1945)
51. Gahan, J. B., Gilbert, I. H., Peffly, R. L., and Wilson, H. G., *J. Econ. Entomol.*, **41**, 795–801 (1948)
52. Gannon, N., and Decker, G. C., *J. Econ. Entomol.*, **48**, 240–42 (1955)
53. Granett, P., Connola, D. P., and Lembach, J. V., *J. Econ. Entomol.*, **44**, 552–57 (1951)
54. Gunther, F. A., and Blinn, R. C., *Analysis of Insecticides and Acaricides* (Interscience Publishers, Inc., New York, N.Y., 696 pp., 1955)
55. Gunther, F. A., and Blinn, R. C., *Ann. Rev. Entomol.*, **1**, 167–80 (1956)
56. Hartzell, A., and Storrs, E. E., *Contribs. Boyce Thompson Inst.*, **16**, 47–53 (1950)
57. Hartzell, A., Storrs, E. E., and Burchfield, H. P., *Contribs. Boyce Thompson Inst.*, **17**, 383–96 (1954)
58. Hewlett, P. S., and Gostick, K. G., *Ann. Appl. Biol.*, **43**, 213–36 (1955)
59. Hornstein, I., Sullivan, W. N., Tsao, C. H., and Yeomans, A. H., *J. Econ. Entomol.*, **47**, 332–35 (1954)
59a. Hoskins, W. M., *Z. Pflanzenkrankh. u. Pflanzenschutz*, **64**, 498–505 (1957)

60. Hoskins, W. M., and Messenger, P. S., *Advances in Chem. Ser.*, No. 1, 93–98 (1950)
61. Hoskins, W. M., Witt, J. M., and Erwin, W. R., *Anal. Chem.*, **24,** 555 (1952)
62. Ishikura, H., and Goto, A., *Nôgyô Gijutsu Kenkyûjo Hôkoku,* [C]No. 4, 165–70 (1954)
63. Ishikura, H., and Ozaki, K., *Nôgyô Gijutsu Kenkyûjo Hôkoku,* [C]No. 4, 171–76 (1954)
64. Ishikura, H., and Ozaki, K., *Nôgyô Gijutsu Kenkyûjo Hôkoku,* [C]No. 5, 81–98 (1955)
65. Jewett, H. H., and Price, W. A., *Kentucky Agr. Expt. Sta., Univ. Kentucky, Bull., No. 571,* 8 pp. (1951)
66. Johansen, C. A., Westlake, W. E., Butler, L. I., and Bry, R. E., *J. Econ. Entomol.*, **47,** 746–49 (1954)
67. Jones, H. A., Campbell, F. L., and Sullivan, W. N., *Soap,* **11**(9), 99, 101, 103, 105, 107, 109 (1935)
68. Jones L. R., and Riddick, J. A., *Anal. Chem.*, **24,** 569–71 (1952)
69. Kartman, L., and Silveira, M. M. da, *J. Econ. Entomol.*, **39,** 356–59 (1946)
70. Kilpatrick, J. W., *Am. J. Trop. Med. Hyg.*, **4,** 758–61 (1955)
71. Klein, A. K., Laug, E. P., Tighe, J. F., Ramsey, L. L., Mitchell, L. C., and Kunze, F. M., *J. Assoc. Offic. Agr. Chemists,* **39,** 242–53 (1956)
72. Kloke, A., *Z. Pflanzenernähr. Düng. Bodenk.*, **63,** 150–54 (1953)
73. Kocher, C., Roth, W., and Trebouse, J., *Mitt. schweiz. entomol. Ges.*, **26,** 1 (1953)
74. Laug, E. P., *J. Pharmacol. Exptl. Therap.*, **86,** 324–31 (1946)
75. Lindquist, A. W., and McDuffie, W. C., *J. Econ. Entomol.*, **38,** 608 (1945)
76. Lindquist, A. W., Jones, H. A., and Madden, A. H., *J. Econ. Entomol.*, **39,** 55–59 (1946)
77. Lindsay, D. R., and Haines, T. W., *J. Econ. Entomol.*, **44,** 104–6 (1951)
78. Linsley, E. G., and MacSwain, J. W., *J. Econ. Entomol.*, **40,** 358 (1947)
79. Linsley, E. G., MacSwain, J. W., and Smith, R. F., *J. Econ. Entomol.*, **43,** 59–62 (1950)
80. Loschiavo, S. R., *Can. Entomologist,* **87,** 246–49 (1955)
81. Ludwick, C. F., and Decker, G. C., *J. Econ. Entomol.*, **44,** 405–18 (1951)
82. Ludwig, D., *Ann. Entomol. Soc. Am.*, **39,** 496–509 (1946)
83. Madden, A. H., Lindquist, A. W., and Knipling, E. F., *J. Econ. Entomol.*, **38,** 265–71 (1945)
84. Maier, P. P., and Mathis, W., *Modern Sanit.*, **7,** 32, 48–53 (1955)
85. Merrell, D. J., and Underhill, J. C., *J. Econ. Entomol.*, **49,** 300–6 (1956)
86. Metcalf, R. L., and Wilson, C. E., *J. Econ. Entomol.*, **38,** 499 (1945)
87. Morris, M. G., *Bull. Entomol. Research,* **41,** 259–88 (1950)
88. Morrison, F. O., *Ann. Rept. Entomol. Soc. Ontario,* **76th,** 18–22 (1945)
89. Nakajima, M., *Mem. Coll. Agr. Kyoto Univ.*, No. 65, 53–61 (1952)
90. Nelson, R. H., *J. Econ. Entomol.*, **42,** 151 (1949)
91. Newman, J. F., *Chem. & Ind. (London),* 617–19 (1954)
92. Nolan, K., & Wilcoxon, F., *Agr. Chem.*, **5,** 53, 74 (1950)
93. Oppenoorth, F. J., and Dresden, D., *Bull. Entomol. Research,* **44,** 395–400 (1953)
94. Pal, R., *Indian J. Malariol.*, **5,** 195–99 (1951)
95. Pal, R., *Indian J. Malariol.*, **6,** 239–50 (1952)

96. Parkin, C. A., and Green, A. A., *Ann. Appl. Biol.*, **30**, 279–92 (1943)
97. Parkin, C. A., and Green, A. A., *Nature*, **155**, 688 (1945)
98. Pimentel, D., Scheardt, H. H., and Norton, L. B., *J. Econ. Entomol.*, **43**, 510–15 (1950)
99. Pimentel, D., Schwardt, H. H., and Norton, L. B., *Soap Sanit. Chemicals*, **27**, 102–3, 105, 112, 114 (1951)
100. Polen, P. B., and Silverman, P., *Anal. Chem.*, **24**, 733–35 (1952)
101. Potter, C., *Ann. Appl. Biol.*, **25**, 836–54 (1938)
102. Pradhan, S., *Bull. Entomol. Research*, **40**, 1–25 (1949)
103. Pradhan, S., *Bull. Entomol. Research*, **40**, 239–65 (1949)
104. Proverbs, M. D., and Morrison, F. O., *Can. J. Research*, [D]**25**, 12–44 (1947)
105. Reid, J. A., *Bull. Entomol. Research*, **41**, 761–77 (1951)
106. Schechter, M. S., and Hornstein, I., *Advances in Pest Control Research*, **1**, 353–447 (1957)
107. Schoof, H. F., and Kilpatrick, J. W., *J. Econ. Entomol.*, **50**, 24–27 (1957)
108. Schwedtfeger, F., *Z. Pflanzenkrankh. u. Pflanzenschutz*, **61**, 9–17 (1954)
109. Siegler, E. H., *J. Econ. Entomol.*, **45**, 449–51 (1952)
110. Simmons, S. W., *Public Health Repts. (U.S.)*, **60**, 917–27 (1945)
111. Sivik, F. P., and Kulash, W. M., *J. Econ. Entomol.*, **49**, 64–65 (1956)
112. Stansbury, R. E., and Dahm, P. A., *J. Econ. Entomol.*, **44**, 45–51 (1951)
113. Storrs, E. E., and Burchfield, H. P., *Contribs. Boyce Thompson Inst.*, **18**, 69–78 (1954)
114. Sullivan, W. N., Hornstein, I., Yeomans, A. H., and Tsao, C. H., *J. Econ. Entomol.*, **48**, 153–55 (1955)
115. Sumerford, W. T., Fay, R. W., Goette, M. B., and Allred, A. M., *J. Natl. Malaria Soc.*, **10**, 345–49 (1951)
116. Sun, J. Y. T., and Sun, Y. P., *J. Econ. Entomol.*, **46**, 927–30 (1953)
117. Sun, Y. P., and Sun, J. Y. T., *J. Econ. Entomol.*, **45**, 26–37 (1952)
118. Sun, Y. P., and Pankaskie, J. E., *J. Econ. Entomol.*, **47**, 180–81 (1954)
119. Sun, Y. P., *Advances in Pest Control Research*, **1**, 449–96 (1957)
120. Suzuki, T., and Toyama, T., *Botyu-Kagaku*, **22**, 283–92 (1957)
121. Takei, S., Koide, M., and Miyajima, S., *Rikagwaku-Kenkyu-jo Ihô*, **9**, 1–5 (1930)
122. Takei, S., and Miyajima, S., *Rikagwaku-Kenkyu-jo Ihô*, **12**, 946–52 (1933)
123. Tattersfield, F., and Potter, C., *Ann. Appl. Entomol.*, **30**, 259–79 (1943)
124. Teotia, T. P. S., and Dahm, P. A., *J. Econ. Entomol.*, **43**, 864–76 (1950)
125. Terriere, L. C., and Ingalsbe, D. W., *J. Econ. Entomol.*, **46**, 751–53 (1953)
126. Tsao, C. H., Sullivan, W. N., and Hornstein, I., *J. Econ. Entomol.*, **46**, 882–84 (1953)
127. Turner, N., and Woodruff, N., *Conn. Agr. Expt. Sta. Bull. No. 512*, 93–97 (1946)
128. Turner, N., and Woodruff, N., *Conn. Agr. Expt. Sta. Bull. No. 524*, 1–34 (1948)
129. Ward, J., and Burt, P. E., *Bull. Entomol. Research*, **46**, 849–68 (1956)
130. Wasserburger, H. J., *Pharmazie*, **7**, 731–34 (1952)
131. Wingo, C. W., *J. Econ. Entomol.*, **47**, 632–35 (1954)
132. Woodruff, N., and Turner, N., *J. Econ. Entomol.*, **40**, 206–10 (1947)
133. Wylie, W. D., *J. Econ. Entomol.*, **49**, 638–40 (1956)

DECIDUOUS FRUIT INSECTS AND THEIR CONTROL[1,2]

By Martin M. Barnes
University of California Citrus Experiment Station, Riverside, California

The principal aspects of recent research in orchard entomology may be presented by reviewing the chief lines of investigation on insects and mites in apple orchards. Research on control of apple insects is more extensive and comprehensive than that on any other tree fruit—in complement to the range and value of the apple orchard as an agricultural enterprise and reflecting local variation in orchard insect problems. In unsprayed orchards, insects and fungi relentlessly lower the probability that marketable fruit may be harvested in quantity. Therefore, almost universally, apple orchards require chemical protection. The insect pests of apple trees are legion, but the few that are widely distributed contribute a semblance of uniformity which can be misleading. It is the entire complex of apple-tree insects and mites, phytophagous and beneficial, and the destructive potential of the pest species composing it which guide the local emphasis in research on control methods and which detail the orchard spray schedule. The pest complex in an apple orchard varies greatly with the circumstance of adapted native species and the accident of distribution of others. This variation exists against a background of diverse requirements in fungus control and the drastic effect of some fungicides upon biological control of insects and mites. He who is aware of the extent of these differences will recognize the need for contrasting emphasis on biological or chemical control in regional research on insects and mite pests of apple orchards.

Of some 700 publications on this subject since the introduction of DDT, those on direct control by chemicals greatly predominate, and emphasis is placed on them rather than on studies on the biology and ecology of apple insects or on the integration of biological and chemical control. Much of the latter research has been admirably and recently reviewed in this series by Ripper (158). Reference to recent biological studies will be made as is convenient to the topic of chemical control, so that the potential significance of the wealth of detail yet uncovered may be implied.

Books produced by deciduous fruit entomologists are intended primarily for local use, but these are, nevertheless, well worth reading by all since they describe varied orchard faunae and reflect different approaches to orchard entomology. Outstanding among these is the third edition of the

[1] The survey of the literature pertaining to this review was completed in July, 1958.

[2] Undefined common names of insecticides used herein are identified chemically in the *Journal of Economic Entomology*, **50**, 226–28 (1957).

book on fruit pests of England by the dean of orchard entomologists, A. M. Massee (132), with its well-condensed chapter on insecticides by Tew. A revised and expanded much-used book by Kotte on fruit pests and their control in Germany has recently been republished (111). In the United States, manuals on the orchard insects of the Pacific Northwest (141), the apple insects of Maine (116), of Connecticut (65), and Pennsylvania (60) reflect different possibilities in presentation. Frost's bulletin (60), for example, deals with insect control only as this is influenced by insect biology, omitting reference to rapidly outdated insecticide practice.

Recent advances in our knowledge of the use of oil sprays on deciduous fruit trees are largely derived from the basic investigations of P. J. Chapman, G. W. Pearce, and A. W. Avens on relationships between composition and insecticidal efficiency of petroleum oils. These comprehensive studies at Geneva, New York, are summarized in a review by E. H. Smith (165) and in other papers of the same symposium. The influence of this research has been widespread in the U.S.A. and has extended to other countries; e.g., superior oils are widely used in Australia, and in Czechoslovakia specifications of local paraffinic oils for plant protection have been developed (112). Though petroleum oils are being replaced in many areas by synthetic insecticides, they remain in secure reserve.

The Codling Moth

The common English name for *Carpocapsa pomonella* (Linnaeus), the codling moth, is derived from the French *Coeur de Lion*. This derivation signifies much to the orchard entomologist who have long fought this versatile pest. Continuing research is essential if we are to keep pace with this adaptable insect [Porter (151)]. Dickson's (46) discovery that photoperiod is the controlling factor in the induction of diapause in the codling moth outranks any single recent discovery in research with this species, and he is to be congratulated. The codling moth may now be easily reared continuously in the laboratory [Dickson *et al.* (47)]. Knipling (110) used this moth as an example when he considered the possibilities of using sexually sterile male insects as an adjunct to other control methods, an example well taken now that the factor of diapause no longer hampers research which involves rearing. Strains of codling moth may be bred continuously in the laboratory, and levels of resistance to insecticides may thus be more readily explored [Barnes (6)].

Dethier's (38) excellent analysis of pioneering studies of codling-moth attractants paves the way for further investigation. We still need much more information concerning the olfactory behavior of the codling moth, both larvae and adults. We still lack a good chemical lure for practical indexing of moth flight, one more specific and constant in attraction, as compared with fermenting lures. Eyer (55) has made progress in this direction, and his technique of impregnation of solids with attractants may be used widely in lure studies with many insect species.

The codling moth is strongly attracted to the blue and violet light

bands of the mercury spectrum, the same bands appearing also to stimulate oviposition. This subject is reviewed and explored by Collins & Machado (25). Mercury light traps have usually been more effective than fermenting lures in indexing moth populations in early season, apparently since light traps are more effective in low populations and since bait traps may chiefly catch moths which have already laid a considerable proportion of their eggs [Groves (78); Hamstead & Gould (85)]. However, in one instance the fermenting bait was reported to be superior to the mercury light in early season [Savary & Baggiolini (160)]. It should be recognized that the attractiveness of fermenting lures is related chiefly to the products of microbiological action and that contrasting results reported with such baits may be related to variation in both substrate and microflora [Eyer (54); Dethier (38)]. The best way to check performance of a light or bait trap against actual moth flight would appear to be with a rotary net [Alexander & Carlson (1)]. Studies with codling-moth attractants and traps in the future will in general show improvement over those of the past, if more attention is given to the matter of adequate replication and randomization, so that the variability encountered in such trials can be dealt with through statistical analysis.

An interesting adaptation of which the codling moth is capable is that of significantly delayed emergence. Armstrong (3) found such a strain to have developed apparently as a result of selection against early larvae by difficulty in entering immature pear fruits, the calyxes of which were blocked with lead arsenate.

And now let us briefly consider an ancient subject—the entrenched opinion that lead arsenate acts solely as a stomach poison. Dickinson & Witman (48) observed that lead arsenate is very repellent to codling-moth larvae. Similarly, Heriot (93) concluded that the effectiveness of residues incorporating lead arsenate was not in small part, but chiefly attributable to repellent qualities. E. H. Smith (167), in extensive studies with *Conotrachelus nenuphar* (Herbst), concluded that lead arsenate effected control chiefly by gustatory repellency. Larvae of a strain of codling moth "resistant" to lead arsenate showed no resistance when arsenical compounds were injected [Haseman & Meffert (90)]. Can genes conferring a chemical sense be sieved to produce a population lacking in response? Dennys (37) observed that sticky or even dry substances may be fed upon by codling-moth adults, apparently by ejection and reabsorbtion of salivary fluid. Can this habit play a role in codling-moth control with modern insecticides? Once again orchard entomology focuses attention on the sensory behavior of insects as worthy of more study and analysis.

Every entomologist who studies the residual activity of insecticides against codling-moth larvae in the orchard should be familiar with a study on the design of such orchard experiments by Newcomer, Dean & Cassil (144). They concluded that the most efficient manner in which such orchard research may be carried out to produce statistically valid results is by the use of single tree plots with replication. Granted, such an experimental

design does not adequately reflect effects upon moths, but such effects are best studied separately.

The codling moth was among the first insects against which DDT was tried in Wiesmann's (186, 187) early studies with this insecticide in Swiss orchards. Steiner *et al.* (178) and Siegler (163) first recorded its superiority to lead arsenate in laboratory studies, and information on its long residual effectiveness in the field against newly hatched larvae of the codling moth was soon obtained (178). In 1944 and 1945 the effectiveness of DDT in the control of the codling moth was well demonstrated, entomologists in the U.S.A., Canada, New Zealand, and Australia chiefly participating [Harman (88); Newcomer (140); Marshall (130); Taylor (182); Caldwell (18); and many others]. With a susceptible strain, it was eventually shown that control with DDT is high so long as the deposit on the fruit is maintained at 7.5 μg./cm.^{2} [Hamilton, Summerland & Fahey (84)].

Hough (98) observed that DDT deposits are toxic to adults, and Childs (23) found that DDT sprays in the orchard reduced bait-trap returns by 94 per cent as compared with lead arsenate when comparisons were made in 8-acre blocks. This illustrates a second stage in testing a codling-moth insecticide and points up the necessity of area-control trials to show complete orchard effects. However, for reasons made explicit by Newcomer *et al.* (144), large-block trials may be grossly misleading unless preceded by a small-plot, well-replicated study.

Many, though not all, reports of early orchard experiments with DDT were accompanied by observations of great increases in populations of mites, i.e., *Panonychus ulmi* (Koch) (178, 182), *Bryobia arborea* Morgan & Anderson (96, 18), and various *Tetranychus* spp. (98, 184). Similarly, *Eriosoma lanigerum* (Hausman) was widely observed to increase (142, 184). Let it be clearly understood, however, that in many major fruit-producing areas of the world these facts were pale in their significance compared with the effectiveness of DDT against codling moth. The ineffectiveness of lead arsenate in the control of the codling moth had burdened apple growers of many regions with as many as 11 cover sprays, followed by ruinously poor control, and a severe fruit residue problem [Cutright (29) and many others]. This had resulted in the decline of the apple industry in many areas, for example, in midwestern and eastern U.S.A. [Steiner *et al.* (177); Palm (146)], the Okanagan Valley in Canada [noted in Dicker & Kirby (44)] and elsewhere. Thus the effectiveness of DDT against codling moth in early trials foreordained its rapid adoption in those apple-growing areas where the intensive use of lead arsenate was routinely resulting in losses of 10 to 60 per cent of the crop, whether in the U.S.A. (88), Australia (106), Canada (130), or central Asia (71). The orchardist forgoes only that fraction of his harvest which economy dictates.

Mite and aphid problems following orchard use of DDT were quickly apparent, but it should be remembered that other major and minor orchard pests of long standing disappeared as significant problems, and many of

these will be noted later herein. Other problems which were to arise in connection with the use of DDT in the apple orchard were not encountered in the earliest experimental work and depended upon wider use for detection. The red-banded leaf roller, *Argyrotaenia velutinana* (Walker), was observed in outbreak accompanying the first use of DDT on a commercial scale in many areas in eastern North America, and the light-brown apple moth, *Austrotortrix postvittana* (Walker), similarly in Australia—these tortricids being more susceptible to lead arsenate than DDT [Chandler (19); O'Loughlin (145)].

Suppression of codling-moth populations in orchards by general adoption of DDT soon made possible the omission of DDT calyx sprays for this insect, and the adoption of as few as 2 to 4 cover sprays (136, 143, 145). The phenomenal relief provided by the general substitution of DDT for lead arsenate in codling-moth control where this insect has one and a half to three generations a year still extends in the great majority of orchards. Still, after intensive selection had occurred (over approximately the following number of generations [estimated]—15 to 16 in Hamilton County, Ohio; 16 at Paracombe, South Australia; and 30 at Soquel, California) apparently genuine failures with DDT were noted [Cutright (32); L. C. Smith (171); Madsen (125)].

Cutright (30) confirmed the existence of DDT tolerance in the Ohio population by well-replicated orchard experiment. Newly hatched larvae from this population proved markedly tolerant to DDT in the laboratory also [Hamilton (81)]. The South Australia population was studied by the Entomology Department of the Waite Agricultural Research Institute and, though detailed laboratory data are not yet published, this population is believed to be highly resistant [L. C. Smith (170, 171)]. Furthermore, a total of 12 DDT sprays at 14-day intervals provided only 56 per cent sound fruit in a replicated trial in which an organic phosphate provided 98 percent undamaged fruit [L. C. Smith & Lohmeyer (172)]. The California population could not be controlled by a well-timed standard DDT schedule in replicated plots [Madsen & Hoyt (128)], and the LC_{50} of this population is four times that of a nonresistant strain in laboratory studies exposing newly hatched larvae to residues on fruit [Barnes (6)]. Thus, without question, the codling moth has locally developed high tolerance to DDT, the population in South Australia being now far more widespread than any other. Apart from the somewhat isolated instances of pronounced DDT failure, there has been more widely observed a tendency for DDT sprays not to provide their customary high degree of protection [Hamilton (81); Glass & Fiori (70)].

Meanwhile, studies had been vigorously pursued with new materials for codling-moth control. Though Dilan was found about equal to DDT and methoxychlor almost so, neither of these materials have significantly replaced DDT, nor were these and other materials structurally similar to DDT effective on DDT-tolerant strains [Glass (68); Hamilton *et al.* (83); Cutright (30); L. C. Smith (170)].

Sy (181) and Hough (99), however, had noted that parathion was initially more effective than DDT, though of shorter residual action, in orchard and orchard-laboratory studies. Driggers (52) had shown that parathion sprays would kill codling-moth larvae after they had entered the fruit. Entomologists at the Gorsem Research Center, Belgium, confirmed this effect and observed significant ovicidal properties of parathion and its high initial effects upon adults (*27*). In an extensive laboratory and orchard study of organic phosphate and other insecticides, Hamilton *et al.* (84) developed the following information concerning a 0.03-per-cent parathion spray: (*a*) 100-per-cent initial effectiveness against larvae, this waning to 84 per cent in 12 days and 57 per cent in 20 days; (*b*) 91-per-cent mortality of eggs; and (*c*) 100-per-cent mortality of adults directly treated. Though its initial effects are high, the limited residual action of parathion impairs its usefulness, especially between generations and against late-season fruit entries [Antongiovanni (*2*); Glass (68)]. It is possible that these deficiencies would be less apparent in large-block trials, where the full effects of ovicidal, larvicidal, and adulticidal action of parathion would be attained. In Germany and Belgium emulsifiable solutions of parathion are widely used alone for codling-moth control (181, *27*). Where improvement is desired over the control provided by DDT alone, parathion may be added at the rate of ⅛ to ¼ of the actual DDT dosage; this has been extensively adopted in practice in the U.S.A. (100, 113).

Diazinon and EPN provide control closely approaching that of parathion (68, 172). Malathion appears to have effects closely similar to parathion upon different stages of the codling moth and to exert equal control in the orchard when employed at two or three times the dosage used for parathion (84, 124). Trithion, Delnav [2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate)], ethion (*O,O,O'O'*-tetraethyl *S,S'*-methylene bisphosphorodithioate), and especially Sevin (*N*-methyl-1-naphthyl carbamate) and Guthion have provided promising results in initial orchard experiments for codling-moth control (124, 128).

DDT substitutes available in quantity for universal use against the codling moth all have broad-spectrum effects. Entomologists in those areas where lead arsenate and natural control have failed to match the destructive potential of the codling moth have shown little inclination in the main to extend their interests beyond the excellent chemical control which they are obtaining, to concern themselves with the effects of their recommendations upon beneficial species. Such concern is clearly unwarranted where broad-spectrum materials must be used. While the destructive potential of the codling moth is the chief factor in such use, also significant is the damage formerly caused by a host of other insect species over a long period of time before the advent of broad-spectrum toxicants (see section entitled "Other Insects," p. 353). Among lead-arsenate and DDT substitutes, however, ryania has offered good performance as a codling-moth insecticide comparing favorably with DDT and without its pronounced side effects [Clancy (24); Hamilton & Cleveland (82)]. World supplies of ryania

would at present scarcely make possible its universal adoption, but this material is gradually being established in apple orchards of some areas because of the distinct local advantage of its selective action and because its orchard use does not involve a residue problem [Patterson & MacLellan (148); Wildbolz & Staub (188); Butz (17)].

Other Moths

An assurgency of the summer fruit tortricid, *Adoxophyes orana* (Fischer von Roeslerstamm), in Belgium and the Netherlands in 1949 and 1950, was accompanied by the discovery of this moth in the ancient horticulture of England and its interception in the U.S.A. [De Jong (36); Groves (77); Bradley (13)]. In Germany and Switzerland it was considered new as a pest and caused considerable damage to foliage and fruit of apple in some areas in the period 1951 to 1953 [Steinhausen (179); Bender (10); Geier (67)]. Hardman (87) has reviewed its bionomics in England where it retains the status of a minor pest. This type of pest outbreak is a constant though unpredictable factor in orchard entomology. In an age of rapid transportation, the problem of the spread of such pests in periods of high population is accentuated. Other recent examples of outbreaks on apple are: *Lithocolletis crataegella* Clemens in Virginia, 1944–45; *Lithocolletis* sp. in California in 1952; and *Callisto geminatella* (Packard) in the mid-Atlantic states in the U.S.A. in 1954–55 [Beckham *et al.* (9); Borden *et al.* (11); Hough & Hill (101)].

As previously noted, the light-brown apple moth, *Austrotortrix postvittana,* increased in importance in coastal Australia after substitution of DDT for lead arsenate. *A. postvittana* is an important pest of apple in Tasmania, and its presence in New Zealand, Hawaii, and southwest England is by introduction [Bradley (14)]. Both this species and *Argyrotaenia velutinana,* which followed a similar pattern in eastern North America, are controlled by either TDE (DDD) or parathion [Graham (73); Taylor (183); Mailloux (129)]. The repetitive use of TDE has brought about the development of resistance to this chemical in *A. velutinana* in 5 to 10 per cent of orchards in New York. Resistance to TDE in populations of *A. velutinana* has been delineated by dosage mortality curves characterizing the heterogeneity of resistant populations [Glass (69)].

The winter moth, *Operophtera brumata* (Linnaeus)—long a severe defoliator of apple trees—is no longer a problem in England when DDT is applied at green cluster (prepink) [Dicker & Briggs (42)]. This treatment also provides protection against at least six other geometrids of apple orchards, notes on the bionomics of which are given by Briggs (16). The winter moth was first recorded in North America in Nova Scotia in 1949, but it may have been present there for many years [Hawboldt & Cuming (91)].

The Woolly Apple Aphid

The woolly apple aphid, *Eriosoma lanigerum* (Hausman), ranks high among insects almost universally infesting apple trees. Fruit growers in

many countries are particularly interested in rootstocks resistant to this insect. The rootstock breeding project begun in England in 1928 has produced clones resistant to woolly apple aphid and these have been named the Malling-Merton (MM) rootstocks [Preston (153)]. It will be several years before they are available in quantity; however, of 14 MM clones selected, at least four are no longer restricted and can be propagated for distribution (15). Massee carried out the woolly-apple-aphid resistance tests and selection of resistant seedlings continues at East Malling (134).

On nursery stocks, Gambrell & Young (62) found that a vigorous BHC spray program checked serious root injury from aphids by preventing aerial infestation. BHC, which is highly toxic to the root systems of many plants, should not be used for root dips (61) or incorporated in soil to which young apple trees are transplanted (164). However, BHC and lindane offer promise in direct soil applications on established nursery stocks (164, 61). BHC is extensively used in prebloom orchard sprays for control of this and other aphids in England (105), Australia (53), and elsewhere. Among organic phosphate insecticides, the development of malathion (142) and parathion (89) for woolly-apple-aphid control was followed by the adoption of malathion as a standard treatment for an investigation of critical timing of orchard sprays against this insect (127). Madsen & Hoyt (127) studied the effects of several organic phosphate insecticides upon the upward and downward migration of woolly apple aphids. Using trap bands on the trunk of apple trees to study aphid movement, they observed that long-term control resulted only from materials which not only controlled aerial colonies, but also, presumably by residual action from trunk deposits, prevented upward movement from subterranean colonies (e.g., Diazinon, Trithion, and Guthion). Preliminary but promising results from the use of trunk treatments with Systox have been reported by Soenen & Vanwetswinkel (174).

Weevils

The plum curculio, *Conotrachelus nenuphar* (Herbst), is a major pest of apples and other deciduous fruits in midwestern and eastern North America. E. H. Smith (168) has developed a continuous rearing procedure for multivoltine strains of this insect and has also explored numerous factors involved in the performance of lead-arsenate residues in its control (166, 167). These studies cannot be reviewed in detail, but they remain an excellent example of the fact that well-designed laboratory studies are necessary to the interpretation of orchard performance of insecticides. Rings & Layne (157) demonstrated radiotracer labeling of the plum curculio with several isotopes, tracing local movements by identifying the release point of a captured labeled insect by radiation characteristics and half-life determinations. Labeled curculios were recovered as long as nine months after release.

The importance of prebloom sprays for control of plum curculio has

been directed to the attention of all by Chandler (20). Dieldrin provides outstanding control of this insect, while EPN, methoxychlor, and parathion are also effective if toxicants with their spectra are needed [Paradis (147); Whitcomb (185); E. H. Smith *et al.* (169)]. A promising means of controlling the plum curculio is by the use of soil treatments. Reasonably low dosages of either aldrin, dieldrin, heptachlor, or chlordane incorporated in the soil have provided control over periods of several years from a single application [Snapp (173); Fluke & Dever (58); and Stelzer & Fluke (180)].

Long a most troublesome pest of Old World orchards, the apple blossom weevil, *Anthonomus pomorum* (Linnaeus), has met with spectacular defeat at the hand of the Swiss chemist. Gone are the days of sack bands and frustration in attempts to reduce losses from this insect. In 1943, DDT was tested in orchards of the Netherlands and Switzerland with promising results [Huysmans (104); Bovey & Martin (12)]. Dicker (39) reported his timely and extensive observations on the bionomics of this weevil in 1946 and also his intensive early study of the utility of DDT in its control (40). Oviposition was shown to begin at fruit bud-burst and DDT sprays to provide excellent control when applied at bud-burst. Massee (134) records that routine treatment with DDT had apparently eliminated the apple blossom weevil by 1948, and there had been no evidence of reinfestation at least by 1956 (all observations pertain to selected East Malling farm plots).

The apple flea weevil, *Rhynchaenus pallicornis* (Say), is readily controlled by DDT as well as by EPN and parathion, whereas formerly this insect could not be readily checked by summer treatment [Cutright (28)]. Similarly, *Rhynchites pauxillus* Germar and *Perperus vermiculatus* Lea are readily controlled by DDT, the biology of the latter species suggesting that trunk sprays would be effective, as is dieldrin against *Brachyrhinus oribricollis* (Gyllenhal) on apple trees [Hallemans (80); Lloyd (123); Shedley (162)].

European Apple Sawfly

Reliable control of yet another important apple pest formerly difficult to suppress—the European apple sawfly, *Hoplocampa testudinea* (Klug)—has been shown to result from the use of any of several broad-spectrum insecticides. Nicotine had provided quite erratic results. Liebster (120) explored the use of BHC or parathion for sawfly control in 1947 and 1948 in northwestern Germany. Davies & Eaton (33) concurrently demonstrated the promise of γ-BHC and parathion as well as chlordane, cautioning against the possible effects of these compounds upon beneficial insects, predators and pollinators alike. With close attention to mode of action and other factors involved in successful use of chemicals against this species, Kirby & McKinlay (108) proposed that failures with nicotine were largely related to its short-term residual effect during periods when cool weather delays hatching. Furthermore, none of the chemicals used (nicotine, DDT,

BHC, aldrin, dieldrin, toxaphene, chlordane, and parathion) exerted their effects through ovicidal action; instead they killed the larvae after eclosion.

In the meantime, Kuenen & van de Vrie (115) had studied the bionomics of the apple sawfly in the Netherlands, observing that oviposition occurs in open blossoms and that BHC gave complete control when applied between petal fall and hatching of the first larvae. Dicker (41) simultaneously observed that oviposition occurred only in open blossoms, and though each provided satisfactory control when applied from petal-fall to 50-per-cent egg hatch, parathion excelled BHC against tunnelling larvae [Dicker & Briggs (43)].

A record of control of European apple sawfly by means of soil surface treatment with BHC has been reported from Russia [Gorjaceva & Beljakova (72)]. Since aldrin, endrin (45), and toxaphene, etc., are effective in spray treatments, soil incorporated insecticides may have a place in European apple sawfly control where horticulture permits.

The European apple sawfly was first recorded in North America from Long Island, New York, in 1939 and Vancouver Island, British Columbia, in 1940 [Pyenson (155); Downes & Andison (50)]. Though not a serious problem in commercial orchards there, damage was first noted in Connecticut in 1944 [Garman (63)] and treatments developed in Europe are now required in the Hudson Valley of New York (138).

The Apple Maggot

Every entomologist working with *Rhagoletis pomonella* (Walsh), the apple maggot or apple fruit fly, may profit from the studies of Hodson (94) on lures and of Lathrop & Dirks (117) on the seasonal cycle of this tephritid. It would appear that the glycine-NaOH lure is a ready tool which could be far more widely exploited in the timing of treatments. Furthermore, Hodson's (95) development of an ammonium-carbonate trap apparently has broad application in surveys for flies of the genus *Rhagoletis*, and tens of thousands of an improved type have been used in California and elsewhere for this purpose. This dry trap has great potential practical utility for tephritid surveys, albeit different lures would be required [Barnes & Osborn (7)].

The problem of control of tephritids is essentially one of area control of adults. Can one depend on field-cage emergence in a central area to tell a grower 50 miles away when to incorporate a material for control of apple maggot? As noted by Garman (64), it would seem most reasonable that some provision should be made for observation on later flight into any given orchard from the outside, as well as on the local emergence within. The glycine-NaOH lure would appear to have utility toward this end in the case of *R. pomonella*. Whatever the practical answer, unless the fly population is being validly indexed and these data are known, one is left wondering about this crucial matter in the interpretation of experimental

results. Research in fruit-fly control is admittedly difficult. Present control methods are apparently to no small extent based on the laboratory studies with insecticides of Dean (35) and the extensive observations and studies of Garman (64).

Later elaboration of results of a research project directed toward biological control of a deciduous fruit insect, plus the intuition and comprehensive approach of Steiner, may be credited with the most striking recent achievements in research on chemical control of tephritids [Hagen (79); Steiner (176)]. These achievements will no doubt be reviewed elsewhere, but deciduous orchard entomology may claim them in their origin.

Other Insects

With foliage sprays of DDT, or with parathion where DDT is ineffective, we now can control the following pests without sprays additional to the regular fungicide or codling-moth spray schedule: *Aspidiotus perniciosus* Comstock, *Lepidosaphes ulmi* (Linnaeus), *Aspidiotus forbesi* Johnson, *Chionaspis furfura* (Fitch), *Aphis pomi* De Geer, *Anuraphis roseus* Baker, *Typhlocyba froggatti* Baker, *Empoasca maligna* (Walsh), *Stictocephala bubalus* (Fabricius), *Lygidea mendax* Reuter, *Plesiocorus rugicollis* Fallèn, *Spilonota ocellana* (Denis & Schiffermüller), *Lithophane antennata* (Walker,) *Lyonetia clerckella* (Linnaeus), *Callisto geminatella* (Packard), *Archips argyrospila* (Walker), *Archips rosana* (Linnaeus), *Scolytus rugulosus* (Ratzburg), *Torymus druparum* Boheman, *Ametastegia glabrata* (Fallen), *Dasyneura mali* (Kieffer), *Thrips imaginis* Bagnall, and many other insect species sufficiently common to be listed in spray schedules [Butz (17), and others]. *Magicicada septendecim* (Linnaeus) may at long last be satisfactorily controlled with yet another broad-spectrum toxicant, Sevin [Graham & Krestensen (74)].

European Red Mite and Other Orchard Mites

Groves's (76) annotated bibliography of literature on the European red mite, *Panonychus ulmi* (Koch), with its concise introductory review by Massee, covers the literature on this species through 1947. The period from 1948 to 1957 has been unusually productive of investigations on all species of orchard mites, and a comprehensive survey of these studies has been presented by Gasser (66), who draws also from the older literature. Lee's (118, 119) investigations on the role of photoperiod, temperature, and nutrition in the evocation of diapause in *P. ulmi* are a model of high professional achievement. Observations on the role of photoperiod in hatching of either winter or summer eggs as influenced by light indicate that further study is needed fully to resolve the factors included, though enough is known that normal hatch may be obtained under suitable artificial conditions [Hueck (102); Becker (8); Kirby & Gambrill (107)].

While noting that other influences may not be excluded, Kuenen (114) observed with *P. ulmi* that egg production was greater on trees in good

condition than on neglected ones. Similarly, Hamstead & Gould (86) noted a positive correlation between high level of N in apple leaves and *P. ulmi* populations per leaf, any possible influence of N on leaf area, however, being unresolved. Considerable interest attaches to the research of Hueck *et al.* (103), who lay claim to an increase in fecundity of *P. ulmi* from the influence of DDT and who postulate that this phenomenon is related to certain critical dosages of this insecticide.

The writer wonders whether that "impertinent questioner," the statistician, has been satisfied in a host of investigations with *P. ulmi*. Clearly, however, such an objection may not be made to the studies of Lienk, Chapman & Curtis (122), who recorded that Cortland apple trees which were severely infested with *P. ulmi* the previous season bore 62 per cent fewer fruits than trees on which infestation had been chemically controlled ($P = 0.01$). Similarly, Preston (152) reports that the greater the leaf injury by *P. ulmi*, the smaller the fruit at harvest ($P < 0.05$). The sample size necessary to estimate size and quality of fruit, growth of apple trees, and per-cent fruit set of apples is discussed by Schultz & Schneider (161). Statistical aspects of chemical control studies with *P. ulmi* were investigated by Freeman & Hall (59), and at East Malling it has been the statisticians who have devised techniques for the design, sampling, and analysis of such experiments, an example worthy of emulation. There appears to be a dearth of statistical studies with application to control experiments with mites. Use of single-tree plots with many replicates would appear to be one of the best workable procedures adopted to date in efforts to deal with the orchard variation encountered in initial screening of acaricides (5, 126). Studies on methods for estimating phytophagous and predaceous orchard mite populations have been accompanied by improvements in techniques in the use of the brushing machine of Henderson & McBurnie (92) and agreement that the use of this equipment is more efficient than other procedures [Morgan *et al.* (139); Chant & Muir (22)].

Space limitations prevent review of the immense amount of information available with respect to the specificity of acaricides among orchard mites. Acaricides have been shown to differ in their effectiveness against different orchard-mite species, and since different mite species may be found simultaneously on the same tree and different species follow one another in seasonal succession, or in annual predominance, the specificity of acaricides has made species identification necessary in selecting materials for mite control [Pritchard & Baker (154); Dosse (49)]. Differences in ovicidal vs. acaricidal action has emphasized timing of treatments as well as selection of chemicals.

Control of *P. ulmi*, and *Bryobia arborea* as well, is currently being concentrated in the use of prebloom ovicides, timing being a critical factor. This is followed in summer by the use of acaricides, preferably those with ovicidal action. Especially in prebloom sprays, the effectiveness of 2,4-dichlorophenyl- or 4-chlorophenyl benzenesulfonate and of chlorbenside is proving a distinct contribution [Kirby *et al.* (109); Madsen & Hoyt (126);

Fjelddalen & Stenseth (56); Reali & Rota (156)]. Usually from petal-fall onward, several materials, notably Kelthane, Chlorbenzilate, Sulphenone, ovex, and Aramite are widely developed, although Aramite is more effective for various *Tetranychus* sp. than for *P. ulmi* (121, 34). Parathion, Systox and EPN are effective in the control of *P. ulmi* from pink stage onward [Downing (51); Armstrong (4)]. The potential uses of Tedion and Amiton [*S*-2-diethylaminoethyl) *O,O*-diethyl phosphorothioate hydrogen oxalate] await full development [Flik (57); Grandori & Rota (75)]. Ethion, Guthion, and Trithion and its 2-5 dichloro analogue, Fenkapton, are currently under development as broad-spectrum organic phosphates with acaricidal action (66, 126).

The disturbing tendency of *P. ulmi* to develop resistance to organic phosphates and to chlorinated phenyl benzenesulfonates must be considered whenever control sprays are routinely required [Cutright (31); Madsen & Hoyt (126)]. The development of resistance may be described as a directed event in that repetitive use of a chemical over many generations may usually be expected to produce it. Orchard entomologists transform these populations. If one environment produces resistance, perhaps another may avert it. While we wait the discovery of means whereby this may be achieved, the search must be pressed for new acaricides. Alternation of chemicals with different modes of action may assist in this problem, but this is difficult to extend into grower practice. The mite resistance factor is currently met in the orchard through the availability of compounds of different chemical structure; e.g., mites resistant to an organic phosphate may be controlled by Kelthane and similar materials.

Chemical Control: A Brief Assessment

The accomplishments of the orchard entomologist have been phenomenal in the field of direct orchard protection with insecticides, and the contributions of these findings to other entomological investigations have been considerable. The view that the entomologist who concentrates upon the study and development of insecticides is a kind of fireman, a devotee of spectacular use of exciting weapons, whose works are destined to defeat by resistance and biotic influences does not appear to be in the realm of provident judgment. There must be a frame of reference for the introduction of an insecticide into practice before we may discover the extent of the factor of resistance to it and before we have any reason to assess the effects of the insecticide against beneficial species. That frame of reference, provided by the chemical control specialist in the orchard, for example, consists of its toxicity, speed and type of action, and residual effectiveness against one or more orchard pests and of the characteristics of its formulations with respect to dilution, tree reaction, and persisting residues. The task of providing this information, which has been exceedingly well done, has required extensive research by many entomologists during a period of rapidly multiplying new pesticides.

The development of information on the parameters of direct use of

chemicals against orchard insects and mites precedes encounters with resistance and imbalance and is, in fact, used to describe these. There must be reasonably extensive use over a period of time before the total effects of a chemical upon orchard arthropod populations are revealed. The development of these materials provides the weapons with which resistance may one day be averted and at least in the meantime defeated, or which, by the accidental grace of specificity, may prove relatively harmless to biotic environmental resistance. The more numerous the insecticides, acaricides, and compatible fungicides for which the primary limits of potential direct use in orchards have been set forth, the greater is the likelihood that some of these may prove to be selective in situations where selectivity may be of value.

The utility of selective insecticides is currently well recognized by the orchard entomologist, especially where the codling moth has one generation a year. The need for the discovery of selective insecticides is issued as a challenge [Massee (133)] from a point of view inspired by one of the earliest (1929) authenticated examples of the disturbance of animal populations by chemicals—the effect of dormant tar-oil sprays upon populations of *Panonychus ulmi* in the apple orchard [Massee & Steer (135)]. If this was the first stimulus for the wide recognition of the effects of chemicals upon arthropod populations, it has indeed endured. The realization of the significance of insecticide resistance, relayed through the insect toxicologist and physiologist into the common fund of man's knowledge concerning the fundamental similarities of cell reaction, may well be said to have originated with the discovery of resistance—by the entomologist observantly working with insecticides in the apple orchard [Melander (137)]. The study of orchard insecticides has in these ways and in a multitude of others generously supplied the entomologist primarily attracted by the heritage of ecology, toxicology, physiology, taxonomy, or biological control with powerful new weapons, new stimuli, and glorious opportunity. His continued devotion to his cause is welcomed.

Meanwhile, it seems futile to face the problem of insecticide resistance or of resurgent pest populations with the conjecture that "the chemical cart has run away with the biological horse" [Solomon (175)]. Nor can we in good reason dismiss the saving of local deciduous fruit industries by the use of DDT, as a "case of fashion overruling common sense (*sic*)" [Holmes (97)] merely because a tortricid problem is created. The orchard entomologist who develops the use of modern insecticides not only recognizes the problems of resistance and resurgence, in fact, he discovers them. He lives with these problems, takes them in stride, and applies varied approaches to their solution. While so doing, he achieves previously unattainable control of insect and mite pests. A change in objectives and a shift from one approach to another do not apply uniquely to the development of insecticides; rather, these are characteristics of progress in all mundane affairs, in which the study of entomology in any manner is obviously included. That such change is apparent in the development of insecticides attests the faithfulness of the record and reflects the productivity of the application of the physical sci-

ences to the problems of biology. Anyone seeking economically to manage the ecology of irrigated apple orchards bearing 20 to 30 arthropod pests in a semiarid environment scant of parasites and predators would soon turn to the only answer in 1958, the routine use of broad-spectrum toxicants [Marshall & Morgan (131)]. It has been implied that such high level of insecticide development in orchard entomology does not represent genuine science [Solomon (175)]. It should be well recognized that in every one of its phases and stages of development the application of chemistry to biology presents just as much scientific achievement and offers just as much scientific challenge and opportunity as the speculative recording of food chains [Solomon (175)] or the pursuit of a more definitive biological study. Among its other aspects, science has to do with the prediction of events. The development of orchard insecticides has surely reached as high a plateau in this regard as any other phase of entomology.

Entomology operates by teamwork and it may well be said that it is needless and futile to imply that any one part is more important than another [Ross (159)]. Let us accept this conclusion and extend it. Even with teamwork, it is purposive tension in the individual which is the root of the solution of any problem. Therefore, we can recognize that the ideal of an impersonally detached truth needs abridgement more to allow for the inherently personal character of the act by which truth is declared. Hence, it is also quite needless and futile to deny the role of controversy in significant progress in any field of science [Polanyi (149, 150)].

There appears little possibility of surcease in the near future from relatively extensive use of insecticides and acaricides in apple orchards of most regions. Though economic in practice, the use of insecticides has been observed to shift emphasis to nonsusceptible species. Among attempts to break this cycle, the potential use of microbial agents provides the orchard entomologist with yet another reason to claim immunity from Alexander's lament that there are no more worlds to conquer.

There is no instance, with any crop, of research leading toward synchronous chemical and biological control progressing to so high a level or coping with so much complexity as that of A. D. Pickett and his associates in the apple orchards of Nova Scotia, previously reviewed (158). Similar studies, in some aspects even more detailed, are underway at East Malling [Collyer (26); Chant (21)]. From a biological point of view, these are the noblest of all wars against orchard insects, since they are based upon sheer biological principle. In these studies, the entomology of apple orchards clearly leads the profession in a synthesis of a new approach to the control of agricultural pests.

LITERATURE CITED

1. Alexander, C. C., and Carlson, F. W., *J. Econ. Entomol.*, **36**, 637–38 (1943)
2. Antongiovanni, E., *Riv. Fruitticult.* **15**, 183–96 (1953)
3. Armstrong, T., *Can. Entomologist.*, **77**, 231–33 (1946)
4. Armstrong, T., *Ann. Rept. Entomol. Soc. Ontario*, **84**, 35–45 (1953)

5. Barnes, M. M., *J. Econ. Entomol.*, **44**, 672–84 (1951)
6. Barnes, M. M., *J. Econ. Entomol.*, **51** (In press, 1958)
7. Barnes, M. M., and Osborn, H. T., *J. Econ. Entomol.*, **51** (In press, 1958)
8. Becker, H., *Anz. Schädlingskunde*, **25**, 116–18 (1952)
9. Beckham, C. M., Hough, W. S., and Hill, C. H., *Virginia Agr. Expt. Sta. Tech. Bull No. 114*, 19 pp. (1950)
10. Bender, E., *Badische Obst- u. Gartenb.*, **5**, 177–78 (1952); abstract in *Z. Pflanzenkrankh. u. Pflanzenschutz*, **60**, 200 (1953)
11. Borden, A. D., Lange, W. H., and Madsen, H. F., *J. Econ. Entomol.*, **46**, 519 (1953)
12. Bovey, P., and Martin, H., *Rev. hort. Suisse romande*, **17**, 8 pp. (1944)
13. Bradley, J. D., *The Entomologist*, **85**, 1–4 (1952)
14. Bradley, J. D., *Bull. Entomol. Research*, **47**, 101–5 (1956)
15. Brase, K. D., *N. Y. State Agr. Expt. Sta. (Geneva, N. Y.) Bull. No. 773*, 41 pp. (1956)
16. Briggs, J. B., *Ann. Rept., East Malling Research Sta., Kent, 1955*, 141–45 (1956)
17. Butz, E. L., *Indiana, Illinois, Kentucky, Ohio and Tenn. Expt. Stas. and U. S. Dept. Agr. cooperating, Purdue Univ. Agr. Ext. Serv. Mimeo ID-28*, 4–9 (1958)
18. Caldwell, N. E. H., *Queensland J. Agr. Sci.*, **5**, 61–76 (1948)
19. Chandler, S. C., *Illinois Agr. Expt. Sta. Bull. No. 519*, 295–330 (1946)
20. Chandler, S. C., *J. Econ. Entomol.*, **46**, 506–9 (1953)
21. Chant, D. A., *On the Ecology and Morphology of some Predacious Laelaptid Mites, with Observations on Other Orchard-Inhabiting Arachnids* (Doctoral thesis, University of London, London, England, 1956)
22. Chant, D. A., and Muir, R. C., *Ann. Rept. East Malling Research Sta. Kent, 1954*, 141–45 (1955)
23. Childs, L., *J. Econ. Entomol.*, **40**, 452 (1947)
24. Clancy, D. W., *Mountaineer Grower*, **25**, 11, 21 (1955)
25. Collins, D. L., and Machado, W., *J. Econ. Entomol.*, **36**, 885–93 (1943)
26. Collyer, E., *J. Hort Soc.*, **28**, 246–59 (1953)
27. *Comité national pour l'etude de la culture fruitiére, Belgique, Rappt. gen., 2me Section, 1950–1952*, 16–22 (1952)
28. Cutright, C. R., *J. Econ. Entomol.*, **43**, 947 (1950)
29. Cutright, C. R., *Ohio Agr. Expt. Sta. Research Bull. No. 720*, 53 pp. (1953)
30. Cutright, C. R., *J. Econ. Entomol.*, **47**, 189–90 (1954)
31. Cutright, C. R., *J. Econ. Entomol.*, **51**, 255 (1958)
32. Cutright, C. R. (Personal communication, 1958)
33. Davies, R. G., and Eaton, J. K., *Ann. Rept. East Malling Research Sta. Kent, 1949*, 146–50 (1950)
34. Dean, F. P., and Newcomer, E. J., *J. Econ Entomol.*, **45**, 1038–42 (1953)
35. Dean, R. W., *J. Econ. Entomol.*, **47**, 479–85 (1954)
36. De Jong, D. J., *Meded. Directeur Tuinbouw*, **14**, 131–50 (1951)
37. Dennys, A. A., *Sci. Agr.*, **22**, 577–83 (1942)
38. Dethier, V. G., *Chemical Insect Attractants and Repellents* (The Blakiston Co., Philadelphia, Pa., 289 pp., 1947)
39. Dicker, G. H. L., *J. Pomol. Hort. Sci.* **22**, 140–52 (1946)
40. Dicker, G. H. L., *Ann Rept. East Malling Research Sta., Kent, 1945*, 140–41 (1946)

41. Dicker, G. H. L., *J. Hort. Sci.*, **28,** 238–45 (1953)
42. Dicker, G. H. L., and Briggs, J. B., *Ann. Rept. East Malling Research Sta., Kent, 1951,* 169–71 (1952)
43. Dicker, G. H. L., and Briggs, J. B., *Ann. Rept. East Malling Research Sta., Kent, 1952,* 151–55 (1953)
44. Dicker, G. H. L., and Kirby, A. H. M., *Ann. Rept. East Malling Research Sta., Kent, 1954,* 163 (1955)
45. Dicker, G. H. L., and Tew, R. P., *J. Hort. Sci.*, **32,** 254–64 (1957)
46. Dickson, R. C., *Ann Entomol. Soc. Am.*, **42,** 533 (1949)
47. Dickson, R. C., Barnes, M. M., and Turzan, C. L., *J. Econ. Entomol.*, **45,** 66–68 (1952)
48. Dickinson, B. C., and Witman, E. D., *J. Econ. Entomol.*, **37,** 43–46 (1944)
49. Dosse, G., *Anz. Schädlingskunde,* **27,** 65–71 (1954)
50. Downes, W., and Andison, H., *Proc. Entomol. Soc. British Columbia,* **39,** 13–16 (1942)
51. Downing, R. S., *Proc. Entomol. Soc. British Columbia,* **49,** 24–26 (1953)
52. Driggers, B. F., *J. Econ. Entomol.*, **43,** 948–49 (1950)
53. *Agr. Gaz. N. S. Wales,* **64,** 36–37 (1953)
54. Eyer, J. R., in *New Mexico Agr. Expt. Sta., 52nd Rept.*, 55–58 (1941)
55. Eyer, J. R., *J. Econ. Entomol.*, **38,** 344–46 (1945)
56. Fjelddalen, J., and Stenseth, C., *Frukt og Baer,* **10,** 77–81 (1957)
57. Flik, H. M., *Rept. Intern. Hort. Congr., 14th Meeting (The Hague-Scheveningen, Netherlands),* **1,** 837–44 (1955)
58. Fluke, C. L., and Dever, D. A., *J. Econ. Entomol.*, **47,** 1115–17 (1954)
59. Freeman, G. H., and Hall, M. J., *Ann. Rept. East Malling Research Sta., Kent,* 102–6 (1955)
60. Frost, S. W., *Penn. State Univ. Agr. Expt. Sta. Bull. No. 535,* 30 pp. (1951)
61. Gambrell, F. L., and Gilmer, R. M., *N. Y. Agr. Expt. Sta. (Geneva, N. Y.) Bull. No. 776,* 21–25 (1956)
62. Gambrell, F. L., and Young, H. C., Jr., *J. Econ. Entomol.*, **43,** 463–65 (1950)
63. Garman, P., *Conn. Agr. Expt. Sta. Bull. No. 488,* 341–44 (1945)
64. Garman, P., *Conn. Agr. Expt. Sta. Bull. No. 604,* 1–20 (1957)
65. Garman, P., and Townsend, G. F., *Conn. Agr. Expt. Sta. Bull. No. 552,* 84 pp. (1952)
66. Gasser, R., *Boll. zool. agrar. e bachicolt., Univ. studi Milano,* **22,** 81–142 (1957)
67. Geier, P., *Rev. romande agr. viticult. et arboricult.*, **9,** 83–84 (1953)
68. Glass, E. H., *J. Econ. Entomol.*, **47,** 1093–1101 (1954)
69. Glass, E. H., *J. Econ. Entomol.*, **50,** 674–76 (1957)
70. Glass, E. H., and Fiori, B., *J. Econ. Entomol.*, **48,** 598–99 (1955)
71. Glusenkow, N. A., *Sad i Ogorod,* No. 7, 34–36 (1953)
72. Gorjaceva, E. P., and Beljakova, M. B., *Sad i Ogorod,* No. 5, 78–9 (1954)
73. Graham, C., *J. Econ. Entomol.*, **42,** 354–56 (1949)
74. Graham, C., and Krestensen, E. R., *J. Econ. Entomol.*, **50,** 713–15 (1957)
75. Grandori, R., and Rota, P., *Boll. zool. agrar. e bachicolt., Univ. studi Milano,* **21,** 53–68 (1955)
76. Groves, J. R., *A Synopsis of the World Literature on the Fruit Tree Red Spider Mite,* Metatetranychus ulmi *(C. L. Koch, 1835), and its Predators, with a Brief Review of the Problem by A. M. Massee, D. Sc., F.R.E.S.* (Commonwealth Inst. Entomol., London, England, 180 pp., 1951)

77. Groves, J. R., *Ann. Rept. East Malling Research Sta., Kent, 1951,* 152–54 (1952)
78. Groves, J. R., *Ann. Rept. East Malling Research Sta., Kent, 1954,* 146–48 (1955)
79. Hagen, K. S., *J. Econ. Entomol.,* **43,** 101–4 (1950)
80. Hallemans, A., *Rept. Intern. Congr. Plant Protect., 1st Meeting,* 265–69 (Heverlé, Belgium, 1946)
81. Hamilton, D. W., *J. Econ. Entomol.,* **49,** 866–67 (1956)
82. Hamilton, D. W., and Cleveland, M. L., *J. Econ. Entomol.,* **50,** 756–59 (1957)
83. Hamilton, D. W., McAlister, H. V., Summerland, S. A., and Fahey, J. E., *J. Econ. Entomol.,* **45,** 462–66 (1952)
84. Hamilton, D. W., Summerland, S. A., and Fahey, J. E., *J. Econ Entomol.,* **47,** 768–75 (1954)
85. Hamstead, E. O., and Gould, E., *J. Econ. Entomol.,* **43,** 724–26 (1950)
86. Hamstead, E. O., and Gould, E., *J. Econ. Entomol.,* **50,** 109–10 (1957)
87. Hardman, J. A., *The Entomologist,* **86,** 264–72 (1953)
88. Harman, S. W., *J. Econ. Entomol.,* **38,** 280–81 (1945)
89. Harris, W. B., *J. Dept. Agr. S. Australia,* **52,** 597 (1949)
90. Haseman, L., and Meffert, R. L., *Missouri Univ. Agr. Expt. Sta. Research Bull. No. 202,* 11 pp. (1933)
91. Hawboldt, L. D., and Cuming, F. G., *Can. Dept. Agr. Bi-Monthly Progr. Rept. for Insect. Invest.,* **6,** 1–2 (1950)
92. Henderson, C. F., and McBurnie, H. V., *U. S. Dept. Agr. Circ. No. 671,* 11 pp. (1943)
93. Heriot, A. D., *Proc. Entomol. Soc. British Columbia,* **40,** 3–8 (1943)
94. Hodson, A. C., *J. Econ. Entomol.,* **36,** 545–48 (1943)
95. Hodson, A. C., *J. Econ. Entomol.,* **41,** 61–66 (1948)
96. Hogan, T. W., and Stephens, R. M., *J. Dept. Agr. Victoria,* **44,** 423–26 (1946)
97. Holmes, E., *Chem. & Ind (London),* 1300–2 (1953)
98. Hough, W. S., *Virginia Fruit,* **33,** 183–92 (1945)
99. Hough, W. S., *J. Econ. Entomol.,* **41,** 207–9 (1948)
100. Hough, W. S., *J. Econ. Entomol.,* **46,** 368–69 (1953)
101. Hough, W. S., and Hill, C. H., *J. Econ. Entomol.,* **49,** 217–19 (1956)
102. Hueck, H. J., *Nature,* **167,** 993–94 (1951)
103. Hueck, H. J., Kuenen, D. J., Den Boer, D. J., and Jeager-Draafsel, E., *Physiol. Comparata et Oecol.,* **2,** 371–77 (1952)
104. Huysmans, C. P., *Tijdschr. Plantenziekten,* **50,** 1–19 (1944)
105. Kearns, H. G. H., in *Science and Fruit,* 217 (Wallace, T., and Marsh, R. W., Eds., University of Bristol, England, 308 pp. 1953)
106. Kemp, H. K., *J. Dept. S. Australia,* **51,** 6–9, 184–86, 238 (1947)
107. Kirby, A. H. M., and Gambrill, R. G., *Ann. Rept. East Malling Research Sta., Kent, 1953,* 175–79 (1954)
108. Kirby, A. H. M., and McKinlay, K. S., *J. Hort. Sci.,* **28,** 170–76 (1953)
109. Kirby, A. H. M., Tew, R. P., and Gambrill, R. B., *Ann. Rept. East Malling Research, Sta., Kent, 1953,* 171–74 (1954)
110. Knipling, E. F., *J. Econ. Entomol.,* **48,** 459–62 (1955)
111. Kotte, W., *Krankheiten und Schädlinge im Obstbau und ihre Bekämpfung, 3rd ed.* (P. Parey, Berlin, Germany, 519 pp., 1958)
112. Koula, V., and Durasova, M., *Sborník Československ. akad. zemědělských věd.,* [A]**27,** 97–106 (1954)

113. Kriner, E. R., Dewey, J. E., and Norton, L. B., *J. Econ. Entomol.*, **45**, 957–65 (1952)
114. Kuenen, D. J., *Tidjschr. Entomol.*, **91**, 83–102 (1948)
115. Kuenen, D. J., and van de Vrie, M., *Tidjschr. Plantenziekten*, **57**, 135–37 (1951)
116. Lathrop, F. H., *Maine Agr. Expt. Sta. Bull. No. 540*, 88 pp. (1955)
117. Lathrop, F. H., and Dirks, C. O., *J. Econ. Entomol.*, **38**, 330–34 (1945)
118. Lees, A. D., *Ann. Appl. Biol.*, **40**, 449–86 (1953)
119. Lees, A. D., *Ann. Appl. Biol.*, **40**, 487–97 (1953)
120. Liebster, G., *Anz. Schädlingskunde*, **22**, 39 (1949)
121. Lienk, S. E., and Chapman, P. J., *J. Econ. Entomol.*, **46**, 1085–86 (1954)
122. Lienk, S. E., Chapman, P. J., and Curtis, O. F., Jr., *J. Econ. Entomol.*, **49**, 350–53 (1956)
123. Lloyd, N. C., *Agr. Gaz. N. S. Wales*, **63**, 90–92, 99 (1952)
124. Lohmeyer, V. K., *J. Dept. Agr. S. Australia*, **61**, 127–29 (1957)
125. Madsen, H. F. (Personal communication, 1958)
126. Madsen, H. F., and Hoyt, S. C., *Calif. Agr.*, **11**, 6, 15–16 (1957)
127. Madsen, H. F., and Hoyt, S. C., *J. Econ. Entomol.*, **50**, 402–6 (1957)
128. Madsen, M. F., and Hoyt, S. C., *J. Econ. Entomol.*, **51** (In press, 1958)
129. Mailloux, M., *Rev. d'Oka agron. méd. vét.*, **28**, 113–22 (1954)
130. Marshall, J., *Proc. Wash. State Hort. Assoc., 42nd Ann. Meeting*, 66–70 (1946)
131. Marshall, J., and Morgan, C. V. G., *Can. Entomologist*, **88**, 1–5 (1956)
132. Massee, A. M., *The Pests of Fruits and Hops*, 3rd ed. (Crosby Lockwood & Son, London, England, 325 pp., 1954)
133. Massee, A. M., *Ann. Rept. East Malling Research Sta., Kent, 1954*, 137–40 (1955)
134. Massee, A. M., *Ann. Rept. East Research Sta., Kent, 1956*, 31 (1957)
135. Massee, A. M., and Steer, W., *J. Ministry Agr. (England)*, **36**, 253–57 (1929)
136. May, A. W. S., *Queensland Agr. J.*, **71**, 325–27 (1950)
137. Melander, A., *J. Econ. Entomol.*, **7**, 167–72 (1914)
138. Mills, W. D., and La Plante, A. H., *N. Y. State Agr. Expt. Sta. (Geneva, N. Y.) Ext. Bull. No. 812*, 47 pp. (1957)
139. Morgan, C. V. G., Chant, D. A., Anderson, N. H., and Ayre, G. L., *Can. Entomologist*, **87**, 189–99 (1955)
140. Newcomer, E. J., *Proc. Wash. State Hort. Assoc., 42nd Ann. Meeting*, 39–52 (1946)
141. Newcomer, E. J., *U. S. Dept. Agr. Cir. No. 270*, 63 pp. (1950)
142. Newcomer, E. J., and Dean, F. P., *J. Econ. Entomol.*, **46**, 54–56 (1953)
143. Newcomer, E. J., and Dean, F. P., *J. Econ. Entomol.*, **46**, 414–19 (1953)
144. Newcomer, E. J., Dean, F. P., and Cassil, C. C., *U. S. Dept. Agr. Bur. Entomol. Plant Quarantine, ET 215* (1943)
145. O'Loughlin, G. T., *J. Dept. Agr. Victoria*, **46**, 442–44 (1948)
146. Palm, C. E., *Advances in Chem. Ser.*, No. 1, 218–22 (1950)
147. Paradis, R. O., *Rept. Quebec Soc. Protect. Plants, 1955*, 88–100 (1956) 25–32 (1955)
148. Patterson, N. A., and MacLellan, C. R., *Ann. Rept. Entomol. Soc. Ontario*, **85**, 25–32 (1954)
149. Polanyi, M., *Brit. J. Phil. Sci.*, **8**, 89–103 (1957)
150. Polanyi, M., *Bull. Atomic Scientists*, **13**, 114–19 (1957)
151. Porter, B. A., *Bull. Entomol. Soc. Am.*, **3**, 4–7 (1957)

152. Preston, A. P., *J. Hort. Sci.*, **29**, 269-77 (1954)
153. Preston, A. P., *J. Hort. Sci.*, **30**, 25–33 (1955)
154. Pritchard, A. E., and Baker, E. W., *Hilgardia*, **21**, 253–72 (1952)
155. Pyenson, L., *J. Econ. Entomol.*, **36**, 218–21 (1943)
156. Reali, G., and Rota, P., *Boll. zool. agrar. e bachicolt., Univ. studi Milano*, **20**, 175–92 (1954)
157. Rings, R. W., and Layne, G. W., Jr., *J. Econ. Entomol.*, **46**, 473–77 (1953)
158. Ripper, W. E., *Ann. Rev. Entomol.*, **1**, 403–38 (1956)
159. Ross, H. H., *Bull. Entomol. Soc. Am.*, **1**, 6–7, 15 (1955)
160. Savary, A., and Baggiolini, M., *Landwirtsch. Jahrb. Schweiz*, **69**, 827–64 (1955)
161. Schultz, E. F., Jr., and Schneider, G. W., *Proc. Am. Soc. Hort. Sci.*, **66**, 36–44 (1955)
162. Shedley, D. G., *J. Dept. Agr. W. Australia*, **5**, 599 (1956)
163. Siegler, E. H., *J. Econ. Entomol.*, **37**, 157–58 (1944)
164. Siegler, E. H., *J. Econ. Entomol.*, **46**, 177–78 (1953)
165. Smith, E. H., *Advances in Chem. Ser.*, No. 7, 3–11 (1952)
166. Smith, E. H., *J. Econ. Entomol.*, **47**, 871–79 (1954)
167. Smith, E. H., *J. Econ. Entomol.*, **50**, 177–83 (1957)
168. Smith, E. H., *J. Econ. Entomol.*, **50**, 187–90 (1957)
169. Smith, E. H., Grainger, M. M., and Avens, A. W., *J. Econ. Entomol.*, **49**, 14–18 (1956)
170. Smith, L. C., *J. Dept. Agr. S. Australia*, **59**, 12–15 (1955)
171. Smith, L. C. (Personal communication, 1958)
172. Smith, L. C., and Lohmeyer, V. K., *J. Dept. Agr. S. Australia*, **60**, 185–87 (1956)
173. Snapp, O., *J. Econ. Entomol.*, **50**, 457–59 (1957)
174. Soenen, A., and Vanwetswinkel, G., *Fruit Belge*, **24**, 1–6, 26–31 (1956)
175. Solomon, M. E., *Chem. & Ind. (London)*, 1143–47 (1953)
176. Steiner, L. F., *J. Econ. Entomol.*, **45**, 838–43 (1952)
177. Steiner, L. F., Arnold, C. H., and Summerland, S. A., *J. Econ Entomol.*, **37**, 29–33 (1944)
178. Steiner, L. F., Arnold, C. H., and Summerland, S. A., *J. Econ. Entomol.*, **37**, 156–57 (1944)
179. Steinhausen, W., *Anz. Schädlinskunde*, **27**, 86–87 (1954)
180. Stelzer, L. R., and Fluke, C. L., *J. Econ. Entomol.*, **51**, 131–33 (1958)
181. Sy, M., *Z. Pflanzenkrankh. u. Pflanzenschutz*, **55**, 29–34 (1948)
182. Taylor, G. G., *New Zealand J. Sci. Tech.*, [A]**27**, 129–33 (1945)
183. Taylor, G. G., *New Zealand J. Sci. Tech.*, [A]**33**, 98–103 (1952)
184. Wason, E. J., *Agr. Gaz. N. S. Wales*, **57**, 427–31 (1946)
185. Whitcomb, W. D., *J. Econ. Entomol.*, **45**, 68–72 (1952)
186. Wiesmann, R., *Schweiz. Z. Obst. u. Weinbau*, **51**, 155–56 (1942)
187. Wiesmann, R., *Schweiz. Z. Obst. u. Weinbau*, **52**, 171–204 (1943)
188. Wildbolz, T., and Staub, A., *Schweiz. Z. Obst. u. Weinbau*, **67**, 32–36 (1958)

SEED TREATMENT AS A METHOD OF INSECT CONTROL[1]

By W. Harry Lange, Jr.
Department of Entomology, University of California, Davis, California

Seed treatment—the application of insecticides, fungicides, growth-regulating materials, trace elements, or other entities to seeds—is rapidly becoming a standard agricultural practice. In fact, the value of fungicide seed treatment has long been recognized. With a decrease, in certain areas at least, of available agricultural lands, and major increases in costs of crop production, an emphasis has been placed upon stand establishment. Proper application of materials to seeds often insures a satisfactory stand, and as an economical insurance control measure, beneficial results need not to be obtained for each crop or season.

Recent reviews have partly covered some aspects of seed treatment. Lilly (122), in his review of soil insects, mentions several of the advantages and disadvantages of seed treatment. A comparison of seed treatment with other methods of control for wireworms and seed maggots was made by Lange, Lane & Stone (116). The status of seed treatment through 1952 was summarized by Fletcher (53). Classic reviews of systemic insecticides in pest control practices and in soil and seed treatments were presented by Ripper (165) and Reynolds (158).

In this review the term "seed" is restricted to true seeds or ovules planted for agricultural purposes; it is not applied to the propagative portions of plants. Contact or fumigant materials on stored grains seem to warrant separate attention, and the subject was covered in a recent review by Parkin (149).

Seed treatment is not a new method of insect control; as David & Gardiner (34) and Ripper (165) point out, the method was thought of as early as A.D. 50 by Junius Columella. It was not until the advent of the use of chlorinated hydrocarbons, lindane in particular, that contact, fumigant, repellent, and limited systemic effects were obtained on certain soil- and foliage-feeding insects. Recent developments in systemic insecticides and fungicides, growth-regulating materials, and trace elements make the future use of seed treatment still more intriguing and useful to the entomologist in solving his many control problems.

FACTORS INFLUENCING SEED TREATMENT

The efficacy of seed treatment is dependent upon a multitude of interacting factors. The many and varied seed-treatment practices now recommended are an indication of the complexity of the problem. For conveni-

[1] The survey of literature pertaining to this review was completed in May, 1958.

ence we can divide relevant factors into insect-independent and insect-dependent categories in an attempt to evaluate successes and failures in the use of seed treatment [Lange, Lane & Stone (116)].

Insect-independent factors include the following: (*a*) seed type; (*b*) seed viability; (*c*) storage period and storage conditions before and after treatment; (*d*) moisture content of seed; (*e*) chemical, and dosage; (*f*) chemical formulations used, particle sizes, etc.; (*g*) compatibility of materials and adjuncts; (*h*) chemical treatment or fumigation prior to treatment; (*i*) method of treating; (*j*) microflora of seed coat and soil; (*k*) soil characteristics and climatic influences (moisture, temperature) at time of planting; and (*l*) planting practices (amount of seed, depth, etc.).

Insect-dependent factors include: (*a*) species of insect; (*b*) diurnal activity of insect in soil; (*c*) type and duration of life history; (*d*) uniformity of brood; (*e*) population of insect; and (*f*) zonal distribution of insect in soil.

Insect-Independent Factors

Seed factors.—The seed type, age of the seed, state of maturity, degree of pericarp injury, extent of frost injury, method of harvesting, and drying methods all play a part in determining seed vitality and the final value derived from seed treatment. Pericarp injury to corn, according to Koehler (103), allows the rapid entrance of soil fungi, and immature seeds are highly susceptible to seedling blight.

The number of seeds per unit weight, type of seed coat, and amount of seed planted per acre also influence seed treatment. In Table 1, for example, the number of seeds per ounce vary from 23.6 for standard lima beans, to 25,175 for lettuce. The nature of the seed coat, and whether it is carried to the surface, or left in the soil, determines control with certain materials. With sugar beets, Lange, Carlson & Leach (114) found that old seed balls treated with lindane affected wireworms several months after planting.

Moisture content of the seed is important in that it can be an indication of substandard quality [Toole (188)]. Seed of high moisture content can be more easily injured by certain chemical treatments. Jones (90) points out that in England periodically occurring high moisture content and also frequent low viability of grain are recurring problems. Seed quality often becomes more important when chemicals are to be applied.

The nature of the seed coat itself is often an important factor in determining the adhesiveness of materials to seeds, and the type of treatment best suited to a particular seed. Many rough-coated seeds as those of sugar beets and spinach, for example, cannot be satisfactorily treated with slurries, but require liquid applications. Small seeds, unattractive to wireworms, many give poor control when treated with lindane [Lange (112)].

Chemicals and formulations used.—The object of seed treatment is to obtain a satisfactory control of the insect, or control or protection from a pathogen, with the minimum amount of chemical and at a rate not

TABLE I

SEED TYPE AND EFFECTIVE RATES OF LINDANE FOR WIREWORM CONTROL IN RELATION TO THE AMOUNT OF SEED PLANTED, SEEDS PER OUNCE, LINDANE PER SEED, AND AMOUNTS APPLIED PER ACRE

Seed	Ounces Lindane per 100 Pounds Seed		Pounds Seed Planted per Acre*	Number Seeds per Ounce†	Deposit of Actual Lindane per Seed (mg.)	Amount Actual Lindane per Acre (ounces)
	75%	100%				
Sugar beets	5.33	4.00	*8*–12	1,644	0.0431	0.320
Tomato	2.66	2.00	0.5	11,482	0.0039	0.01
Cotton‡	2.66	2.00	25	226	0.1570	0.50
Corn	1.33	1.00	6–*12*	*110*–120	0.1613	0.12
Peas	1.33	1.00	60–*100*	85	0.2088	1.0
Okra	1.33	1.00	8	450	0.0394	0.080
Cucumbers	1.33	1.00	1–*4*	1,077	0.0164	0.04
Cantaloupes	1.33	1.00	1–*4*	1,276	0.0139	0.04
Watermelon	1.33	1.00	4	312	0.0568	0.04
Lettuce	1.33	1.00	1–*1.5*	25,175	0.0007	0.015
Carrot	1.33	1.00	3.25	23,417	0.0007	0.0325
Barley	1.33	1.00	80–*100*	852	0.0208	1.0
Milo	1.33	1.00	2–*5*	937.5	0.0189	0.05
Sunflower	1.33	1.00	3–*7*	187.5–562.5:*250*	0.0710	0.07
Onion	1.33	1.00	*4*–5	9,667	0.0018	0.04
Spinach	1.33	1.00	*8*–15	2,835	0.0063	0.08
Bean (baby bush limas)	0.66	0.5	45–*55*	71	0.1250	0.275
Beans (bush, snap, all others except limas)	0.66	0.5	30–*60*	113	0.0785	0.30
Oats	0.66	0.5	75–90	795.2	0.0111	0.45
Sudan grass	0.66	0.5	3–*15*	3,408	0.0026	0.075
Rye	0.66	0.5	80–*100*	1,136	0.0078	0.50
Safflower	0.66	0.5	30–*40*	500–812.5:*562.5*	0.0157	0.20
Alfalfa	0.66	0.5	2–*15*	14,200	0.0006	0.075
Soybean	0.66	0.5	30	170–*369*	0.0240	0.15
Radish	0.66	0.5	10	4,000	0.0022	0.05
Cabbage	0.66	0.5	2	8,930	0.0009	0.01
Beans (standard large limas)	0.33	0.25	100–*120*	23.6	0.1880	0.30
Beans (Fordhook large limas)	0.33	0.25	100–*120*	28.5	0.1556	0.30
Wheat	0.33	0.25	80–*100*	710	0.0062	0.25

* Numbers in italics refer to average pounds planted per acre.

† Numbers in italics refer to average number seeds per ounce.

‡ Acid de-linted cotton.

phytotoxic to the seedling or to later plant growth. Some chemicals are more phytotoxic than others. The formulation used often influences damage.

The recent case of the apparent incompatibility of Thimet 44D, a systemic insecticide, with certain mercurial fungicides indicates the difficulties encountered in combining protective materials [Erwin & Reynolds (47)]. That the so-called "inerts" influence adhesiveness of fungicides to seeds is brought out by Fitzgibbon (52). He found that a definite crystalline form and a particle size of 10μ is desirable. Srivastava (175) found finely ground, solvent-free, wettable powders of aldrin, lindane, and heptachlor to be desirable, together with the addition of methyl cellulose. Kantack (94, 95) used Carbowax 6000, a water-soluble polyethylene glycol, as a carrier for systemic insecticides for control of the wheat curl mite, *Aceria tulipae* (Keifer).

The use of "safeners," such as Aroclor 5460 (a chlorinated terphenyl), with lindane seed treatments has been tested by Harwood, Nelson & Telford (71) and by Lange (112). Finlayson & Handford (51) attributed retarding of germination of onion seeds with emulsions of aldrin and dieldrin to the solvent and not the insecticides.

Methods of treating.—Chemicals can be applied to seeds in several ways: (*a*) dust or dry applications; (*b*) slurries; (*c*) liquid fixation, in which dusts that have already been applied to the seeds are stuck firmly with fluids; and (*d*) liquid treatment, in which the insecticides, usually solutions, are sprayed on the seed or used as a seed dip [Lange, Carlson & Leach (115)]. The best method for a given situation will depend upon the seed type, the quantity of seed to be treated, the pest or pathogen to be controlled, the chemical to be used, and the availability of equipment.

For the control of the seed-corn maggot, *Hylemya cilicrura* (Rondani), slurries give better controls than dust applications [Lange *et al.* (113); Hofmaster & Nugent (76)]. Liquid combinations of soluble fungicides and insecticides are receiving attention because of the ease with which they can be applied.

Storage.—With the usual contact materials (lindane, aldrin, dieldrin, and heptachlor) used at the proper dosages, Hofmaster & Nugent (76) found no detrimental effects to treated seeds related to injury or control of seed-corn maggots or to seed decay organisms, after a three- to four-month storage period at optimum storage conditions. With the exception of dieldrin, however, most of the insecticides lost their effectiveness for maggot control after a one-year storage period. Lange (112) found no effect of a one-year storage of sugar-beet seed treated with lindane, but 50 per cent of the insecticide's effectiveness in controlling the Pacific Coast wireworm, *Limonius canus* Leconte, was lost. The adverse effects of storage of treated seed under high temperatures was demonstrated by Howe, Schroeder & Swenson (79) and Hofmaster & Nugent (76). McEwen *et al.* (126) report that when lima-bean seed is treated with dieldrin or lindane at the rate of 0.25 ounce per bushel 50 to 75 per cent of this dosage can be lost and still obtain good

control. They found Diazinon more residual than dieldrin or lindane, and after eight months' storage they found no appreciable difference between lindane and dieldrin at the dosage rates cited above.

Soil factors.—Soil type and texture, amount of organic matter, and soil temperature and moisture may play a part in determining seed-treatment effectiveness. Lindane damage to sugar-beet seed in England is reported to occur more often in sandy soils than in other types [F. G. W. Jones (92)]. In California, lindane injury to seeds is more frequently associated with low temperatures and high moisture, conditions favorable to pre-emergence seed decay organisms [Lange, Carlson & Corrin (113)].

The importance of moisture in seed germination is reviewed by Doneen & MacGillivray (39). Steudel (181), in testing systemic materials for aphid control on sugar beets in Germany, records phytotoxic symptoms in dry soil.

Planting practice and plantability.—The amount of seed planted per acre, type of planter used, depth of planting, and the effect of the seed treatment or rate of seeding all enter into value obtained from seed treatment. Disyston on cotton seed was found to decrease the rate of planting (10). De Zeeuw *et al.* (35) discovered that slurry treatment of beans with aldrin, lindane, dieldrin, chlordane, and heptachlor increased the friction and decreased planting rate.

Newlin (143), in discussing the place of seed treatment as a means of controlling soil insects on hybrid seed corn in Iowa, states: "We believe that the reductions in planting rate of 5 to 15 per cent which occur when insecticides are added to the fungicide seed treatment more than offset any advantage that might be received from the insecticide."

Although materials such as graphite and silicones [Natti & Schroeder (140)] have been added to seed-treatment materials to increase flowability, the subject apparently needs further research. Ward (192) found that cotton seed coated with a mixture of 65-per-cent ground feldspar and 35-per-cent fly ash, or montmorillonite clay, can be more easily seeded, but he received a lower rate of emergence and a lower percentage germination.

Microflora of soil and seed.—A "predisposition" of seed to decay when insecticides alone are placed on them has been recorded by several investigators. Lange, Carlson & Leach (114) and Leach *et al.* (119) in 1949 and 1950 trials with Fordhook lima beans and other seeds demonstrated the value of combination insecticide-fungicide materials applied simultaneously as slurries to the seed. Others who have shown the value of combination treatments are Lange, Carlson & Corrin (113), Lange *et al.* (117), Howe, Schroeder & Swenson (79), Lange, Carlson & Leach (115), Leach *et al.* (118, 119), and Hofmaster & Nugent (76).

Laboratory tests with lima beans under controlled temperature and moisture conditions indicated that the "predisposing" effects of lindane applied at a nonphytotoxic level were obtained only in natural soil infested with *Pythium ultimum* Trow, but not in pasteurized soil to which *Pythium ultimum* alone was added. In fact, in sterile soil lindane on the seed acted as a

depressant of decay, according to comparison with untreated seed. It appears that lindane on the seed in natural soil interferes with an antibiotic or other inhibiting entity or entities or with some complicated synergistic relationship with other soil microorganisms which ordinarily keep *Pythium* under control [Lange (112)].

Not only is the soil microflora important in determining results of seed treatment, but the microflora of the seed, other than pathogenic organisms, may play a role in producing antibiotic substances. In an examination of certified bean varieties, Schnathorst (174) found that 65 per cent of the discolored seeds yielded six species of fungi and 11 species of bacteria, whereas only 21.5 per cent of the apparently normal seeds yielded bacteria. In extracts of the coats of seeds inoculated with *Trichoderma viride* Persoon, *Penicillium frequentans* Westling, or *Penicillium gladioli* McCollouch & Thom, and sown in soil, the seed coats contained antibiotics identified as gliotoxin, frequentin, and gladiolic acid, respectively [Wright (195)].

Insect-Dependent Factors

Species.—The species of insect often determines the effectiveness of seed treatment. Tests with wireworms by Lange, Carlson & Leach (114) indicated that *Drasterius dorsalis* Say (reported as *Aeolus* sp.) was the most susceptible, followed in order of their decreasing susceptibility to lindane, by the Pacific Coast wireworm, *Limonius canus* Leconte, the sugar-beet wireworm, *Limonius californicus* Mannerheim, and *Anchastus cinereipennis* Eschscholtz. Differences in the efficacy of seed treatments can often be attributed to the species of insect involved—even in members of the same genus.

Biological differences.—For many soil insects seasonal and diurnal activity may determine the effectiveness of treatment. With contact insecticides for wireworm control, it was noted that there occurs a diurnal movement of wireworms in the soil and that effectiveness is correlated with their activity in the seed zone [Lange (112)]. Whether a soil insect has a single generation a year, or takes several years to complete a cycle can be an important consideration.

In California, differences in the overlap of generations of the seed-corn maggot, *Hylemya cilicrura* (Rondani), are believed to explain discrepancies in control with seed treatments [Lange (112)]. The zonal distribution in the soil is important, as many soil insects may feed at a level above or below the seed and thus render the treatment ineffective.

Populations.—In general, seed treatment is not considered by most investigators to be as effective as general broadcast, row, strip, or topical treatments, or still other methods, if populations are high. Even with such typical soil insects as wireworms, this may not always be the case, and the final evaluation of seed treatment with systemic insecticides and systemic fungicides remains to be determined in most instances.

INSECTS CONTROLLED

Nonsystemic Insecticides

Wireworms.—The place of seed treatment in the control of wireworms depends among other things upon the economics of the crop involved, the species, effectiveness, and the ability of commercial seed producers and handlers to work seed treatment into their established methods of operation. Soil treatments, row treatments with or without fertilizers, fumigation, or other measures are sometimes of a more permanent nature; or seed treatments may be used as an adjunct to these methods of control.

The development of seed-treating techniques for wireworm control has made a tremendous impact on agriculture in the United Kingdom. D. P. Jones (90) states that "although it would be difficult to provide conclusive evidence, the probability is that the introduction of insecticidal seed treatments has, in fact, been the main factor in overcoming the wireworm menace."

In California over 30 different kinds of seeds have been treated for wireworm and seed-corn-maggot control with lindane, dieldrin, heptachlor, endrin, or aldrin, usually in combination with fungicides [Lange, Carlson & Leach (115), and see Table 2]. In addition, cotton seed is being extensively treated with Thimet and a fungicide, although the final value of such treatment remains to be determined. The additional effect of Thimet on *Rhizoctonia* has given an added impetus to the use of this treatment [Erwin & Reynolds (47)].

The value of insecticide treatment of seed corn in Iowa will illustrate some of the problems involved. In February, 1957, Strong (183) surveyed 338 seed-corn producers located in 10 states and found that: in 1954, 6.13 per cent used insecticides on their seeds; in 1955, 14.3 per cent; and 1956, 27.7 per cent. The expected 1957 percentage was 36.7 per cent. Dieldrin was almost universal as a selected insecticide. Of the companies that do not treat seeds, 64.9 per cent sell planter-box insecticides, whereas only 18.5 per cent of the treating companies do so.

Experiences with dieldrin seed treatment in Iowa during 1956 and 1957 have persuaded Newlin (143) that the disadvantages of reduced plantability and difficulties in the disposal of unused treated seed outweigh any value received. In 1956 his survey of 94 fields indicated an average stand increase of 3.5 per cent, and 40 per cent of the fields had a significantly higher stand; in 1957, a stand increase of 3.0 per cent was recorded for 124 fields, and 35 per cent had a higher stand (a stand increase of 4 to 5 per cent was significant).

The question involved here, then, is whether it pays to treat all seed annually for preventive wireworm- or other soil-insect control, when it is difficult to justify the over-all value. In California one sugar-beet company during the period from June 1, 1950, through March 31, 1958, has spray treated 3,015,195 pounds of seed with excellent results and no records of

TABLE II

RATES OF COMBINATION INSECTICIDE-FUNGICIDE MIXTURES TO USE IN THE TREATMENT OF CERTAIN VEGETABLE AND FIELD CROP SEEDS IN CALIFORNIA

Seeds	Suggested Insecticide in Ounces per 100 Pounds of Seed*		Suggested Fungicide to Use with Insecticides†
	Lindane 75%	Dieldrin, Aldrin, Endrin or Heptachlor 75%	
Alfalfa, clover, and other small-seeded legumes	0.66	—	Thiram, dichlone
Barley	1.33	1.00–2.00	Ceresan M or other organic mercuries
Beans, all except large limas	0.66	0.66–1.33	Thiram, chloranil, captan
Beans, large limas	0.33	1.33	Thiram, chloranil, captan
Beets, chard	5.33	5.33	Dichlone, captan, thiram
Broccoli, cabbage, turnip, other crucifers	0.66	0.66	Thiram
Carrots	1.33	0.66	Thiram, chloranil
Cantaloupes, cucumbers, watermelons, squash	1.33	0.66	Thiram, dichlone, captan
Corn	1.33	0.33–0.66	Thiram, chloranil, dichlone, captan
Cotton	2.66	1.00–2.00	Ceresan M, captan, panogen
Flax	—	—	Ceresan M
Grasses, sudan, blue, etc.	1.33	0.66	Thiram, chloranil, captan
Lettuce	1.33	0.66	Thiram
Oats, rye	0.66	0.66	Ceresan M or other organic mercuries
Okra	1.33	—	Chloranil, thiram
Onion	1.33	2.00	Thiram
Peas	1.33	0.66	Dichlone, thiram, chloranil
Pepper	1.33	1.33	Thiram
Rice	—	—	Thiram, dichlone, sodium hypochlorite
Safflower, sunflower	0.66	0.66	Thiram, captan, organic mercuries
Sorghum	0.66	0.66	Thiram, ceresan M, dichlone
Soybean	0.66	0.66	Chloranil, thiram, captan
Spinach	1.33	2.00	Dichlone, captan, thiram
Tomato	2.66	2.00	Ceresan M, thiram
Wheat	0.33–0.66	0.66	Ceresan M, or other organic mercuries

* Slurry treatment, except for treatment of certain seeds such as beets and spinach which require dusts or sprays.

† Rates depend upon seed to be treated; proprietary insecticide-fungicide mixtures are available, as are new formulations of certain fungicides.

phytotoxicity to seed of good viability [Kloor (102)]. The treatment of lima-bean seed in California by combination insecticide-fungicide mixtures has made replanting almost a thing of the past, and in some areas it is used as an adjunct to other soil or strip treatments [Lange, Lane & Stone (116)].

In Oregon, Morrison (138) reports that the Great Basin wireworm, *Ctenicera pruinina noxia* (Hyslop), is present in practically every grainfield and may reduce the stand by 30 per cent. With the stooling of the plants, however, reductions in yield are not credited to wireworms. For the same insect in the state of Washington, Nelson, Telford & Harwood (142) suggest seed treatment with aldrin, heptachlor, dieldrin, BHC, or lindane.

The early use of seed treatments involving repellents, Paris green, arsenicals, strychnine, etc. is adequately reviewed by others and will not be fully discussed here [see Thomas (186, 187) and Srivastava (175)], and later developments are reviewed by Lange (111), Lange, Lane & Stone (116), and Potter, Healy & Raw (152). Fernald in 1909 (48) coated seeds with strychnine, coal tar, and Paris green with negative results; he was followed in 1911 by Johannsen & Patch (87), who coated corn seed with tar and Paris green, lead arsenate, tobacco dust, and slaked lime. Masaitis (129, 130) dusted oat and wheat seeds with arsenicals and with mercuric chloride. Woodworth (193) made an early study of the failure of baits poisoned with arsenicals to kill wireworms.

The use of benzene hexachloride, and later the essentially pure gamma isomer, lindane, as a seed treatment had more or less parallel developments in the United Kingdom, Canada, and California [Jameson, Thomas & Woodward (86); Arnason & Fox (13); King, Arnason & Manson (98); and Lange, Carlson & Leach (114)].

The development of seed dressings in the United Kingdom for the control of *Agriotes* spp. has been reviewed by D.P. Jones (90), but space does not allow full consideration of the subject. He reports that the plowing of permanent pastures during the 1939 to 1945 World War resulted in wireworm populations as high as 5 to 10 million per acre. The discovery of BHC at the I.C.I. laboratories at Jealott's Hill resulted in trials with the chemical as broadcast, combine drilling, and seed dressings. The marketing of an essentially pure γ-BHC preparation was the result of these trials, and commercial seed dressings were used during 1948 and 1949 [Jameson, Thomas & Woodward (86); Jameson, Thomas & Tanner (85)].

The problem of γ-BHC toxicity came to the front in 1951 [Jameson & Callan (84)] and led in the United Kingdom to trials with aldrin and dieldrin. The 1954 to 1955 research of the Fernhurst Research Station of Plant Protection Limited [D. P. Jones (90)] indicated that γ-BHC was on the whole more effective than aldrin or dieldrin, but the nature of the phytotoxicity meant that reasonable care should be exercised in dosage rates and seed handling.

Work on sugar-beet seed treatment in the United Kingdom resulted in a recommendation similar to the method used in California—approximately

0.27-per-cent γ-BHC on the seed [F. G. W. Jones & Humphries (93), and D. P. Jones (89)]. Phytotoxicity was observed in 1954 and 1956, but the reason for damage under particular conditions is still being investigated [Jones & Humphries (93), and F. G. W. Jones (92)].

The value of seed treatment for corn has been investigated by Dogger & Lilly (38), Starnes & Filmer (180), Kulash (105, 106, 107), Kulash & Munroe (110), Bigger & Blanchard (18), Duffield (40), Bigger (17), Lilly (120, 121), Lilly & Gunderson (123), Starks & Lilly (178), Arnold & Apple (14), Apple (11), and Apple, Strong & Raffensperger (12). There is some difference of opinion on the value of seed treatment for corn, and some of the difficulties have already been mentioned.

Bigger (17) found a 36-per-cent control of wireworms of the genera *Melanotus* and *Conoderus* with lindane seed treatment. In recent corn work, Apple, Strong & Raffensperger (12), attempting control of the wheat wireworm, *Agriotes mancus* (Say), and of *Melanotus* sp., obtained a 5.8-per-cent increase in stand and a 5.7-per-cent increase in yield by using 0.1 and 0.25 ounces of heptachlor, dieldrin, or aldrin per bushel. This amounted to from 25.7 to 52.7 per cent of the benefits received from a soil treatment of 1.5 pounds of heptachlor per acre.

On beans seed treatment has been used to advantage by several investigators, including Lange, Carlson & Leach (114, 115), Starnes & Filmer (180), and Apple, Strong & Raffensperger (12). Apple *et al.* obtained a 47.8-per-cent average mortality of the eastern field wireworm, *Limonius agonus* (Say), on baby lima beans, using 0.5 ounce of lindane, dieldrin, heptachlor, or aldrin per 100 pounds of seed when evaluated 18 days after planting.

On wheat, Hastings & Cowan (72) reported the use of "Mergamma" (40-per-cent lindane and 2-per-cent phenyl mercury urea) against *Athous* sp., and Nelson, Telford & Harwood (142) have reported the control of *Ctenicera pruinina noxia.*

The California use of BHC and lindane for seed treatment of sugar beets began with studies in 1946 and led to the widespread use of a combination insecticide-fungicide [Lange (111), and Lange, Carlson & Leach (114)]. Treatment of sugar-beet seed has been greatly facilitated by the development of new spray treaters [Kepner & Leach (97); Kloor (100, 101); and Walters (191)]. Laboratory and isotope-tagged tests with the newer seed-treatment materials have been carried out by Kulash & Munroe (109), Kulash (108), and Long (124), and Long & Lilly (125).

Seed-corn maggot.—Seed treatment for control of the seed-corn maggot, *Hylemya cilicrura* (Rondani) has in general given excellent results, particularly on beans. Combination insecticide-fungicide mixtures and slurries have proved better than insecticides alone or dust combinations. Under conditions in Great Britain, however, no satisfactory controls have been obtained [D. P. Jones (90)].

The earliest work with lindane as seed treatments was conducted by

Brooks & Anderson (24), Dill & Odlund (36), and Ristich & Swardt (167), although Headlee (73) and Turner (189) had considered seed treatments. Floyd & Smith (54) used BHC, chlordane, and DDT in the fertilizer, and Elmore (43) developed a row method of application. Combination fungicide-insecticide mixtures were suggested in 1951 by Lange, Carlson & Corrin (113) and Howe & Schroeder (78). General recommendations for treatment with these combinations followed [Lange, Carlson & Leach (115); Van Dinther (190); Elmore (44, 45); Frost, Anderson & Elmore (55); Hofmaster & Nugent (76); Miller & Dustan (135); Ditman, Cox & Kantzes (37); Miller & McClanahan (136); Nugent & Hofmaster (144); and Natti *et al.* (141)].

In southern Ontario, Miller & McClanahan (136) obtained excellent control of the seed-corn maggot affecting peas, corn, beans, and cucurbits with seed coatings of 0.4 ounce of heptachlor, dieldrin, or lindane combined with 1.5 ounces of thiram or captan. The possibilities of using seed treatments on soybeans for seed-corn-maggot control were explored by Starks & Lilly (179), who found that heptachlor, toxaphene, lindane, and dieldrin were compatible with the soybean bacterial inoculant *Rhizobium leguminosarum* Frank. Strong (184) developed a refined technique for testing the phytotoxicity of insecticides to corn seeds and found that a dieldrin seed treatment as low as 0.5 ounce per bushel prevented seed-corn-maggot development in treated pea seeds. The phytotoxicity and loss of lindane on stored bean seed has resulted in a trend toward the use of dieldrin, or in some cases aldrin, or heptachlor [Anonymous (2, 6)].

In 1957 to 1958 tests [Lange (112)], a number of materials have given control superior to that of lindane, aldrin, endrin, dieldrin, or heptachlor. These materials include Guthion, Trithion, Korlan [*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate], Diazinon, Ethion, and the systemics Thimet, Di-syston [*O,O*-diethyl *S*-2-(ethylthio) ethyl phosphorodithioate], Bayer 25198, and Bayer 25141. Dieldrin has given better control of root attack than lindane. Endrin controlled seed springtails, *Onychiurus* spp., where dieldrin did not give protection, if maggot and springtail damage occurred simultaneously. The use of phosphate materials for seed treatments on corn, soybeans, and sorghum has been explored by Mitchell (137).

Onion maggot.—A calomel seed treatment was suggested in 1929 for the control of the onion maggot, *Hylemya antiqua* (Meigen) [Glasgow (59)]. In 1942 Matthewman, Dustan & Davis (131) found that calomel was not highly effective against onion maggot at Ottawa, but when supplemented with other treatments, its combinations were more effective than any of the other materials singly. Experiments in the interior of British Columbia in 1950 and 1951 [Finlayson & Handford (51)] demonstrated that DDT could be substituted for calomel seed treatment, but the amount of DDT necessary interfered with the flowability of seed. This problem led to the development of the use of dieldrin when Finlayson (50) found that 0.5 ounce per pound of seed gave good control, was not phytotoxic, and gave high yields of

onions. Miller & McClanahan (136) effectively controlled the onion maggot with a seed dressing of 2 ounces of thiram and 1 ounce of heptachlor or dieldrin per pound of seed. Rawlins (154) has found that Thimet and Disyston compare favorably with aldrin and other chlorinated hydrocarbons.

Wheat bulb fly.—An endemic fly through Northern Europe and the U.S.S.R., the wheat-bulb fly, *Leptohylemyia coarctata* (Fallen), is becoming more important with an increase in mechanization and the use of weakly tillering wheat varieties [Jones (90)]. Dual-purpose dressings using lindane and dieldrin are now employed in the epidemic areas [Gough & Woods (63); Potter (151); and Gough (62); and Fidler & Webley (49)].

Other anthomyid flies.—Soil treatments, dips, or row treatments have been more successful than seed treatments in the control of the cabbage maggot, *Hylemya brassicae* (Bouché) and the turnip maggot, *Hylemya floralis* (Fallen). Glasgow (60) treated cabbage seed with calomel. Some work by King *et al.* (99) indicated poor control of root maggots attacking rutabagas.

Carrot rust fly.—Seed treatments have effected some control of the carrot rust fly, *Psila rosae* (Fabricius). Handford (65, 66, 67) in British Columbia obtained excellent control in 1952 tests with aldrin soil treatments for the first generation, but only fair control on the second generation. Seed treatments gave poor control. Trials in 1953 demonstrated the value of aldrin dust or furrow treatments, and 1955 tests gave control of the fly with a seed treatment of "Gammasan" (a proprietary seed treatment material containing γ-BHC) at 1.5 ounces per 3 pounds of seed (4.5 ounces per acre). Fulton & Handford (58) and Harris & Rawlins (69) obtained better controls with soil treatments. The trials of Rawlins & Harris (155) and Hellqvist (74) demonstrated the value of seed treatments with γ-BHC, aldrin, and heptachlor. Salkeld & Scott (173) obtained good control with foliage sprays and seed treatments with parathion.

In Great Britain Jones (90) points out that J. W. R. Jenkins was the first to demonstrate the possibility of seed treatment for carrot-rust-fly control and next came critical work by J. D. Thomas and D. W. Wright. The off-flavors or "flattening" of flavor of carrots has curbed the use of lindane, even though it has been more effective than aldrin or dieldrin.

Frit fly.—The frit fly, *Oscinella frit* (Linnaeus), causes annual crop losses of about £5 million in the United Kingdom [Jones (90)]. Seed-treatment experiments at Fernhurst have not been entirely satisfactory, and investigations indicate that the problem is similar to that of the wheat bulb fly—a matter of relationships between drilling time and the onset of insect attack. Fidler & Webley (49) reported excellent control on oats with seed treatments of γ-BHC, aldrin, and dieldrin. Izotova *et al.* (83) used schradan at 0.2 to 1.0-per-cent concentration as a seed soak with good results.

Flea beetles.—Flea beetles in the genera *Phyllotreta, Chaetocnema,* and *Longitarsus* have been controlled in Great Britain with combined mercurial γ-BHC seed dressings, as a result of early work by J. R. W. Jenkins and

work at Fernhurst by Plant Protection Ltd. [Jones (90)]. The systemic nature of γ-BHC is demonstrated in this work as flea beetles are controlled on the seed leaves and first and second true leaves. The systemic nature of BHC has been shown by Bradbury & Whitaker (21). In France, Bonnemaison & Jourdheuil (19) controlled *Psylliodes chrysocephala* (Linnaeus) by treating rape seed with BHC, lindane, parathion, and aldrin.

Seed-feeding beetles.—The seed-corn beetle, *Agonoderus lecontei* Chaudoir, *Agonoderus comma* Fabricius, and *Clivina impressifrons* LeConte have been controlled with seed treatments of parathion, lindane, chlordane, dieldrin, heptachlor, and aldrin [Johnson (88)].

Other insects.—Wright & Buxton (194) found lindane seed dressings effective on the cabbage seed-stalk curculio, *Ceutorhynchus quadridens* (Panzer). The pygmy mangold beetle, *Atomaria linearis* Stephens, was controlled with γ-BHC seed treatments [Jones & Humphries (93)]. The rice water beetle, *Lissorhoptrus orysophilus* Kuschel (= *simplex* Say) was controlled with preplant seed treatments of lindane, aldrin, dieldrin, and Thimet [Bowling (20)]. Chafer grubs, *Schizonyncha* sp., were controlled by γ-BHC seed treatment [Tarr (185)].

Eden & Arant (42) partially controlled the southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber, with lindane seed treatment. False wireworms, *Eleodes* spp., were controlled by Daniels (32) and Srivastava (175). The thief ant, *Solenopsis molesta* (Say), was controlled with lindane, aldrin, and heptachlor [Srivastava (175) and Srivastava & Bryson (176)]. Seed springtails, *Onychiurus* spp., have been controlled on lima beans with 1 ounce of endrin per 100 pounds of seed used with a suitable fungicide [Lange (112)]. White grubs in wheat (*Cyclocephala* spp., and *Lachnosterna* spp.) were not controlled with dieldrin seed treatment [Burkhardt (25)]. Jones *et al.* (91) found aldrin or heptachlor seed treatments effective in controlling the sugar-beet root maggot, *Tetanops myopaeformis* (Roeder). On the other hand, Callenbach, Gojmerac & Ogden (26) found seed treatments ineffective against the same insect.

Systemic Materials

Adequate reviews of this aspect by Ripper (165) and Reynolds (158) permit limitation of the present discussion of systemic materials applied to seeds. Some materials—lindane, for example—possess contact, repellent, systemic, and vapor action when applied to seeds, but in other cases there is no sharp distinction between systemic and nonsystemic action.

A number of workers have shown that systemic materials applied to seeds can be taken up in quantities sufficient to cause insecticidal action [Ripper, Greenslade & Hartley (166); Ivy, Iglinsky & Rainwater (81); Chao (27); Bronson & Dudley (23); Ashdown & Cordner (15); Reynolds, Anderson & Swift (160); David & Gardiner (34); and Harries & Valcarce (70)]. Fundamental investigations on the metabolism of demeton (Systox), the nature of the residues, uptake of Di-syston, and the use of radiotracer

techniques in following the metabolites were made by Metcalf *et al.* (134), Fukuto *et al.* (56), March *et al.* (128), Fukuto *et al.* (57), and Reynolds, Metcalf & Winton (162).

Systemic materials on seeds have the advantage that the materials are translocated early in the growth of the plant, thereby controlling aphids, thrips, and similar insects and giving protection before side dressings or topical treatments can ordinarily be made. Clark, Johnson & Mattson (29) point out the value of application of a systemic at planting time.

Since many systemics have the disadvantage of possessing high mammalian toxicity and phytotoxicity, they are, for seed treatments, often concentrated on activated carbon [Ivy (80); Ashdown & Cordner (15); and Anonymous (9, 10)]. Systemics are also applied to seeds as seed soaks [Harries & Valcarce (70), David & Gardiner (34), Banerjee & Basu (16)].

Cotton insects.—The use of Thimet and Di-syston on cotton seeds has controlled a wide range of insects. A recent U.S.D.A. summary [Anonymous (8)], gave a 4-to-6-week period of control for the following pests: thrips; aphids; spider mites; cotton leaf perforator, *Bucculatrix thurberiella* Busck (limited control); flea beetles; leaf miners; darkling beetles; false wireworm adults; cotton leafhopper, *Psallus seriatus* (Reuter); white flies (some effect); and the boll weevil, *Anthonomus grandis* Boheman (uneven control). Other general discussions indicate the value of Thimet or Di-syston for cotton-seed treatment [Anonymous (3, 4)]. Good results with schradan, Thimet, and Di-syston against aphids were obtained by most investigators [Hopkins, Fye & Walker (77), and Almeida (1)]; and thrips were controlled in early season growth from 4 to 9 weeks [Robertson & Arant (169); Reed (156); Reed & Arant (157); Robertson (168); and Hanna (68)]. Research on systemic insecticides conducted since 1949 in Texas [Parencia (145); Parencia, Davis & Cowan (147, 148); Stanley & Breeland (177); and Parencia, Cowan & Davis (146)] has demonstrated the value of seed treatment with systemics for early season cotton-insect control. Robertson & Arant (169) controlled the cotton fleahopper, *Psallus seriatus* (Reuter), the onion thrips, *Thrips tabaci* Lindeman, tobacco thrips, *Frankliniella fusca* (Hinds), and the flower thrips, *Frankliniella tritici* (Fitch), with seed treatments. Other investigators have carried out fundamental work on the movement of systemics in cotton plants, including Mostafa *et al.* (139), Ivy, Scales & Gorzycki (82), and Merkel *et al.* (132).

Reynolds *et al.* (161) applied systemic insecticides to seeds of cotton, alfalfa, and sugar beets at time of planting. He obtained effective control on cotton of thrips, *Frankliniella* sp., cotton aphid, *Aphis gossypii* Glover; reduced populations of the southern garden leafhopper, *Empoasca solana* DeLong; controlled white flies, *Trialeurodes abutilonea* (Haldeman), leafminers, *Liriomyza* sp., darkling ground beetles, *Blapstinus* sp., the black cutworm, *Agrotis ypsilon* (Rottemburg); reduced the beet armyworm, *Laphygma exigua* (Hübner); and controlled the beet leafhopper, *Circulifer tenellus* (Baker). Radiotracer studies demonstrated that the highest con-

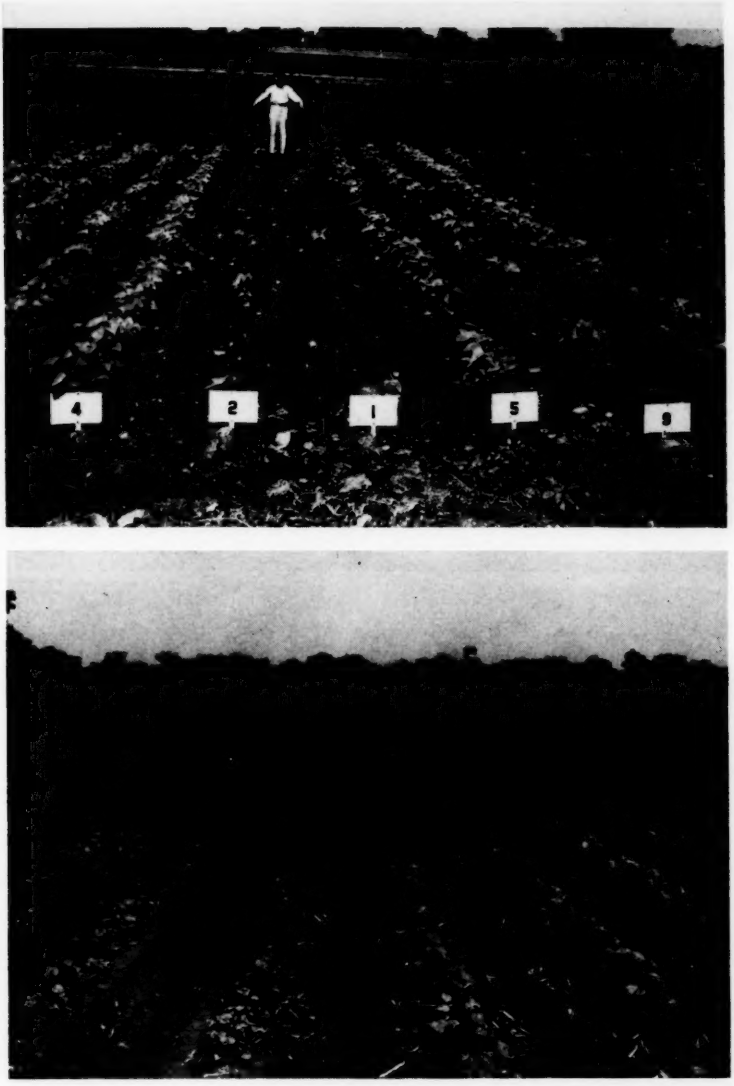


FIG. 1. *(Upper)* Plots showing effect of seed treatments: (4) dieldrin plus thiram; (2) thiram alone; (1) no treatment; (5) dieldrin alone; (9) lindane alone; next row, lindane plus thiram. *(Photograph courtesy of Entomology Department, New York State Agricultural Experiment Station, Geneva, N. Y.) (Lower)* Difference in growth and appearance resulting from thrips control afforded by a seed treatment of cotton of one pound per acre of Thimet at Waco, Texas: left of center, untreated; right of center, treated. *(Photograph courtesy of United States Department of Agriculture)*

FIG. 2. *(Upper)* Value of seed treatment for control of the seed-corn maggot: left, untreated; right, treated with slurry of Bayer 25198 plus thiram. *(Lower)* Effect of overdosage of lindane on sugar-beet seedlings: (1, 2) no treatment; (3, 4, 5) treated with lindane at 3 times normal rate. *(Photograph courtesy of California Agricultural Experiment Station, Davis, Calif.)*

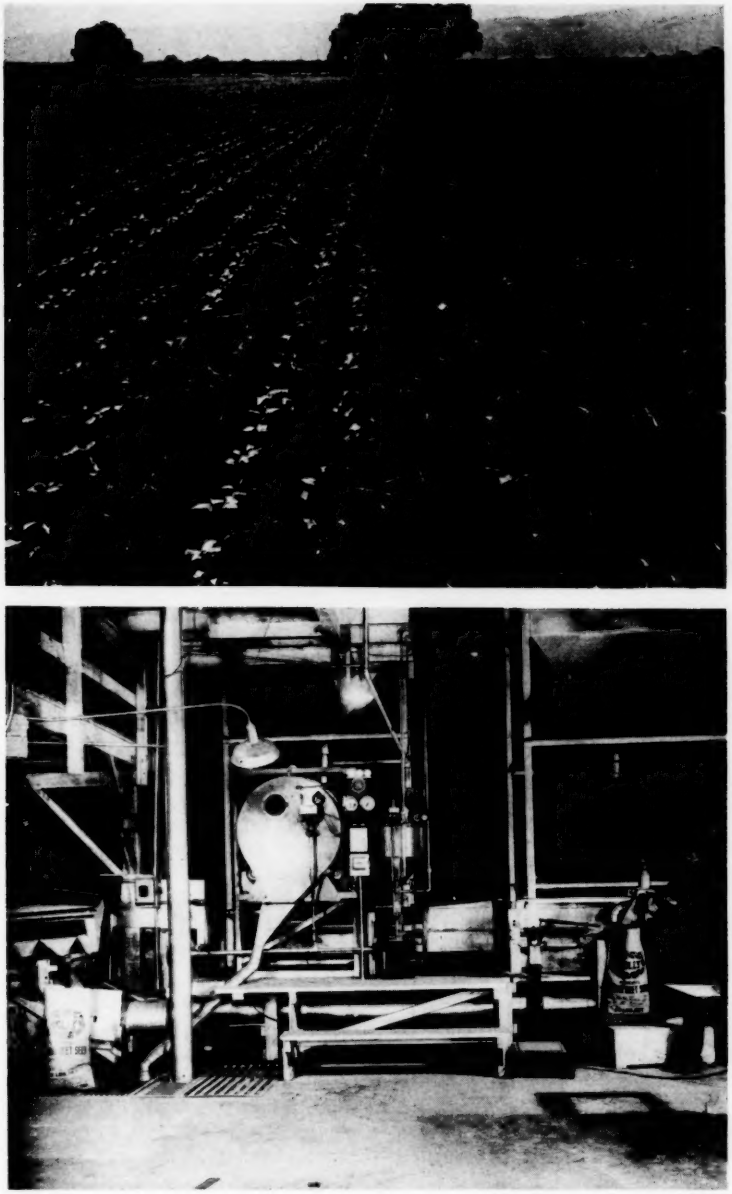


FIG. 3. *(Upper)* Value of seed treatment for control of the seed-corn maggot, Sunnyvale, California: left of center, dieldrin plus captan; right, fungicide alone. *(Photograph courtesy of California Agricultural Experiment Station, Davis, Calif.)* *(Lower)* New spray treater for sugar-beet seed applying lindane, fungicide, and dye. *(Photograph courtesy of Holly Sugar Corporation)*

centrations of systemics, following seed treatment, occur in the cotyledons and that the toxicants are not translocated in substantial amounts to other plant parts. Seed treatments with Thimet and Di-syston were not effective against the lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller) [see Reynolds, Anderson & Andres (159)].

Other pests.—Systemic materials have been effective against the beet leafhopper, *C. tenellus* [Hills, Harries & Valcarce (75)]. The use of dimefox [*bis*(dimethylamino)fluorophosphine oxide], para-oxon (diethyl *p*-nitrophenyl phosphate), and sodium fluoroacetate as seed treatments against *Phaedon cochleariae* Fabricius was demonstrated by David & Gardiner (33). Seed soaked for 8 hr. in a mixture containing 42-per-cent schradan controlled the paddy borer, *Schoenobius incertulus* (Walker) in West Bengal [Banerjee & Basu (16)]. White tip of rice caused by a nematode, *Aphelenchoides besseyi* Christie, was controlled with systemics on rice seeds by Cralley & French (30).

In chemical control studies of the wheat curl mite, *Aceria tulipae* (Keifer), Thimet and Am. Cyanamid 12009 gave good control when used on seed at from 0.125 to 0.5 pound technical material per bushel [Kantack (94, 95)].

Steudel (181) controlled the spinach leaf miner, *Pegomya hyoscyami* (Panzer), with systemics applied to seeds. Anthomyid seed maggots—the seed-corn maggot, *Hylemya cilicrura* (Rondani), and the onion maggot, *Hylemya antiqua* (Meigen), have been controlled in the same manner [Lange (112), and Rawlins (154)]. Thimet seed treatment has also controlled the potato flea beetle, *Epitrix cucumeris* (Harris), the pea aphid, *Macrosiphum pisi* (Harris), the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), the Mexican bean bettle, *Epilachna varivestis* Mulsant, and grasshoppers on wheat [Anonymous (9)].

Control of virus diseases.—The control of insect-borne plant diseases by seed treatment with systemic insecticides has shown some promise, and a great deal of work is still to be done [Kramer (104), and McLean (127)].

MATERIALS COMBINED WITH INSECTICIDES

The value of combining fungicides with insecticides for a dual-purpose treatment of seeds has already been pointed out. Recent advances indicate, also, the possibility of combining insecticides, both systemic and nonsystemic, with supplementary materials such as growth-promoting substances, micro-amounts of chemical elements, and systemic fungicides.

Growth-promoting materials.—Gibberellic acid, a metabolic product of the fungus *Gibberella fujikuroi* (Sawada), induces multiple growth and physiological responses in a number of plants. Stowe & Yamaki (182) have reviewed the history and physiological action of gibberellins. They point out that time of seed germination is reduced by applications of gibberellins, and that they must correspond in their action to naturally occurring compounds in higher plants—hence they are auxins, albeit atypical ones.

The agricultural uses for gibberellins have not been fully explored. Ergle & Bird (46) found that gibberellic acid weakened the hypocotyl of cotton, causing poor stands in compacted soil. The hypocotyl broke in the crook region and left the seedling without cotyledons. The possible agricultural benefits of gibberellic acid as a seed treatment are summarized by Merritt (133), who suggests use on cotton, soybeans, peas, beans, and small grains.

Micro-amounts of chemical elements.—The use of small amounts of certain chemicals applied to seeds has not been thoroughly explored. Popov (150) achieved a stimulation of seeds by treatment with manganese and cobaltous salts. The application of zinc, zinc sulfate, and sodium chloride gave favorable results on corn seed, according to Rafaila & Tusa (153). Zinc sulfate on corn seeds gave a 6.5- to 13.8-per-cent yield increase.

Systemic fungicides.—The combination of systemic fungicides with systemic insecticides would seem to offer a virgin field for future seed-treatment investigations. A recent review by Brian (22) points out that a number of antibiotics are translocated in plants; these include streptomycin, chloramphenicol, chlortetracycline, griseofulvin, mycetin, neomycin, oxytetracycline, penicillin, pleocidin, streptothricin, and viomycin. Kendrick & Zentmyer (96) report advances in the control of soil fungi with antibiotics. The antifungal properties of certain antibiotics have been demonstrated in the control of many plant diseases [Anonymous (5, 7); Gottlieb *et al.* (61); Crowdy & Wain (31); Gregory *et al.* (64); Chinn (28); Rhodes (163); and Dye (41)].

EFFECT ON WILDLIFE

The treatment of seeds with chemicals has sometimes harmed wildlife. The use of some of the newer organophosphorus materials may cause considerable avian and rodent mortalities if seed is not handled properly. Rudd & Genelly (172), in relation to seed treatment, state:

> The degree of actual hazard depends upon the amount and toxicity of seed treated, on wildlife populations and food habits, and on manner of seed dispersal. Aerial sowing, which is now widely practiced, makes available more seed than surface application.

The same authors (171) report the effect of DDT-treated rice seed on the avian populations of rice fields and (170) point out the repellent nature of seed treated with lindane to pheasants. Repellency of seeds is an important agricultural consideration. The use of the alpha isomer of chloralose as a narcotic on seeds to prevent damage from certain obnoxious birds is an example of the possibilities of seed treatments [Ridpath & Burton (164)].

ACKNOWLEDGMENTS

Special acknowledgment goes to H. T. Reynolds for access to manuscripts prior to publication, A. P. Arnason for extensive assistance in Canadian developments, and D. Price Jones for use of a documented history of

the development of seed treatment in the United Kingdom. The author is indebted to J. W. Apple and F. E. Strong for data on corn treatments, illustrations, and an unpublished manuscript; to R. H. Handford and D. G. Finlayson for confidential reports; Herbert Knutson for a copy of a doctorate thesis; P. S. Chapman for the photograph in Fig. 1 (*Upper*); R. F. Harwood for unpublished records; W. M. Kulash for illustrations; Owen Newlin for confidential data on seed treatment of corn; C. R. Parencia for illustrations (Fig. 1, *Lower*); to J. H. Bigger for a report on seed treatment in Illinois; and to L. A. Kloor, Jr., for the photograph in Fig. 3 (*Lower*), and data on sugar-beet seed treatment.

LITERATURE CITED

1. Almeida, P. R. De., *Biológico*, **23**, 145–56 (1957)
2. Anonymous, *Your Crops*, **2**, 1, 3 (1954)
3. Anonymous, *Cyanograms*, **4**, 2–5 (1955)
4. Anonymous, *Cyanograms*, **5**, 18–21 (1956)
5. Anonymous, *J. Agr. Food Chem.*, **4**, 832 (1956)
6. Anonymous, *Your Crops*, **5**, 1, 3 (1957)
7. Anonymous, *J. Agr. Food Chem.*, **5**, 725–26 (1957)
8. Anonymous, *Agr. Research (U.S.)*, **5**, 10 (1957)
9. Anonymous, *Thimet Tech. Manual* (American Cyanamid Co., New York, N. Y., 63 pp., 1957)
10. Anonymous, *Tech. Release* (Chemago Corp., New York, N. Y., 4 pp., 1958)
11. Apple, J. W., *Proc. N. Central Branch, Entomol. Soc. Am.*, **11**, 49–51 (1956)
12. Apple, J. W., Strong, F. E., and Raffensperger, E. M., *J. Econ. Entomol.* (In press, 1958)
13. Arnason, A. P., and Fox, W. B., *Can. Dept. Agr., Sci. Serv.*, 6 pp., (1948)
14. Arnold, E. W., and Apple, J. W., *J. Econ. Entomol.*, **50**, 43–45 (1957)
15. Ashdown, D., and Cordner, H. B., *J. Econ. Entomol.*, **45**, 302–7 (1952)
16. Banerjee, S. N., and Basu, A. C., *Bull. Entomol. Research*, **48**, 299–303 (1957)
17. Bigger, J. H. (Personal communication, 1958)
18. Bigger, J. H., and Blanchard, R. A., *J. Econ. Entomol.*, **48**, 255–60 (1955)
19. Bonnemaison, L., and Jourdheuil, P., *Compt. rend. acad. agr. France*, **40**, 491–93 (1954)
20. Bowling, C. C., *J. Econ. Entomol.*, **50**, 450–52 (1957)
21. Bradbury, F. R., and Whitaker, W. D., *J. Sci. Food Agr.*, **7**, 248–53 (1956)
22. Brian, P. W., *Ann. Rev. Plant Physiol.*, **8**, 413–26 (1957)
23. Bronson, T. E., and Dudley, J. E., Jr., *J. Econ. Entomol.*, **44**, 747–50 (1951)
24. Brooks, J. W., and Anderson, L. D., *J. Econ. Entomol.*, **40**, 220–28 (1947)
25. Burkhardt, C. C., *J. Kansas Entomol. Soc.*, **28**, 60–63 (1955)
26. Callenbach, J. A., Gojmerac, W. L., and Ogden, D. B., *Proc. Am. Soc. Sugar Beet Technologists*, **9**, 300–4 (1958)
27. Chao, S. T., *Nature*, **166**, 909–10 (1950)
28. Chinn, S. H. F., *Can. J. Microbiol.*, **1**, 118–24 (1954)
29. Clark, E. L., Johnson, G. A., and Mattson, E. L., *J. Agr. Food Chem.*, **3**, 834–36 (1955)
30. Cralley, E. M., and French, R. G., *Phytopathology*, **42**, 6 (1952)
31. Crowdy, S. H., and Wain, R. L., *Ann. Appl. Biol.*, **38**, 318–33 (1951)
32. Daniels, N. E., *Texas Agr. Expt. Sta. Progr. Rept. No. 1793*, 2 pp. (1955)
33. David, W. A. L., and Gardiner, B. O. C., *Ann. Appl. Biol.*, **41**, 261–70 (1954)
34. David, W. A. L., and Gardiner, B. O. C., *Ann. Appl. Biol.*, **43**, 594–614 (1955)
35. DeZeeuw, D. J., Andersen, A. L., Merrill, L. G., and Zurakowski, W., *Mich. State Univ., Mich. Agr. Expt. Sta. Quart. Bull.*, **37**(2), 204–17 (1954)
36. Dill, L. E., and Odlund, M. L., *Market Growers' J.*, **78**, 16 (1949)
37. Ditman, L. P., Cox, C. E., and Kantzes, J. G., *J. Econ. Entomol.*, **48**, 688–93 (1956)
38. Dogger, J. R., and Lilly, J. H., *J. Econ. Entomol.*, **42**, 663–65 (1949)
39. Doneen, L. D., and MacGillivray, J. H., *Plant Physiol.*, **18**, 524–29 (1943)
40. Duffield, P. C., *J. Econ. Entomol.*, **45**, 672–74 (1952)

41. Dye, D. W., *New Zealand J. Sci. Technol.*, [A]**37**, 458–61 (1956)
42. Eden, W. C., and Arant, F. S., *Alabama Agr. Expt. Sta. Leaflet No. 34*, 4 pp. (1953)
43. Elmore, J. C., *U. S. Dept. Agr. Bur. Entomol. Plant Quarantine*, ET279, 3 pp. (1950)
44. Elmore, J. C., *J. Econ. Entomol.*, **46**, 1054–59 (1953)
45. Elmore, J. C., *U. S. Dept. Agr. Leaflet No. 370*, 2 pp. (1954)
46. Ergle, D. R., and Bird, L. S., *Plant Disease Reptr.*, **42**, 320–23 (1958)
47. Erwin, D. C., and Reynolds, H. T., *Plant Disease Reptr.*, **42**, 174–76 (1958)
48. Fernald, H. T., *J. Econ. Entomol.*, **2**, 279–80 (1909)
49. Fidler, J. H., and Webley, D. P., *Nature*, **178**, 1480 (1956)
50. Finlayson, D. G., *Can. J. Plant Sci.*, **37**, 252–58 (1957)
51. Finlayson, D. G., and Handford, R. H., *Can. J. Agr. Sci.*, **34**, 385–88 (1954)
52. Fitzgibbon, M., *J. Soc. Chem. Ind. (London)*, **62**, 8–11 (1943)
53. Fletcher, F. W., *Down to Earth*, **8**, 16–19 (1952)
54. Floyd, E. H., and Smith, C. E., *J. Econ. Entomol.*, **42**, 908–10 (1949)
55. Frost, M. H., Jr., Anderson, L. D., and Elmore, J. C., *J. Econ. Entomol.*, **40**, 1040–45 (1954)
56. Fukuto, T. R., Metcalf, R. L., March, R. B., and Maxson, M. G., *J. Econ. Entomol.*, **48**, 347–54 (1955)
57. Fukuto, T. R., Wolf, J. P., III, Metcalf, R. L., and March, R. B., *J. Econ. Entomol.*, **49**, 147–51 (1956)
58. Fulton, H. G., and Handford, R. H., *Can. Dept. Agr. Sci. Serv. Pub. No. 939*, 4 pp. (1955)
59. Glasgow, H., *J. Econ. Entomol.*, **22**, 335–40 (1929)
60. Glasgow, H., *J. Econ. Entomol.*, **27**, 303–8 (1934)
61. Gottlieb, D., Bhattacharyya, P. K., Carter, H. E., and Anderson, H. W., *Phytopathology*, **41**, 393–400 (1951)
62. Gough, H. C., *Agr. Rev. (London)*, **1**(10), 45–47 (1956)
63. Gough, H. C., and Woods, A., *Nature*, **174**, 1151–53 (1954)
64. Gregory, K. F., Allen, O. N., Riker, A. J., and Peterson, W. H., *Phytopathology*, **42**, 613–22 (1952)
65. Handford, R. H., *Ann. Rept. Comm. Hort. Research Can. Hort. Council, Ottawa* (1953)
66. Handford, R. H., *Ann. Rept. Comm. Hort. Research Can. Hort. Council, Ottawa* (1954)
67. Handford, R. H., *Ann. Rept. Comm. Hort. Research Can. Hort. Council, Ottawa* (1956)
68. Hanna, R. L., *J. Econ. Entomol.*, **51**, 160–63 (1958)
69. Harris, E., Jr., and Rawlins, W. A., *Bull. Entomol. Soc. Am.*, **2**, 27 (1956)
70. Harries, F. H., and Valcarce, A. C., *J. Econ. Entomol.*, **50**, 120–22 (1957)
71. Harwood, R. F., Nelson, W. L., and Telford, H. S., *J. Econ. Entomol.*, **50**, 702–3 (1957)
72. Hastings, E., and Cowan, T. E., *J. Econ. Entomol.*, **47**, 597–99 (1954)
73. Headlee, T. J., *Rept. Dept. Entomol. N. J. State Agr. Expt. Sta.*, **40**, 437–42 (1920)
74. Hellqvist, H., *Rev. Appl. Entomol.*, [A]**45**, 40 (1957)
75. Hills, O. A., Harries, F. H., and Valcarce, A. C., *J. Am. Soc. Sugar Beet Technologists*, **9**, 124–28 (1956)

76. Hofmaster, R. N., and Nugent, T. J., *Virginia Truck Expt. Sta. Bull. No. 116,* 31 pp. (1956)
77. Hopkins, A. R., Fye, R. E., and Walker, R. L., *J. Econ. Entomol.,* **51,** 100–2 (1958)
78. Howe, W. L., and Schroeder, W. T., *Farm Research,* **17,** 10–11 (1951)
79. Howe, W. L., Schroeder, W. T., and Swenson, K. G., *N. Y. State Agr. Expt. Sta. (Geneva, N. Y.) Bull. No. 752,* 34 pp. (1952)
80. Ivy, E. E., *Agr. Chem.,* **7,** 44–45, 121, 123 (1952)
81. Ivy, E. E., Iglinsky, W., Jr., and Rainwater, C. F., *J. Econ. Entomol.,* **43,** 620–32 (1950)
82. Ivy, E. E., Scales, A. L., and Gorzycki, L. J., *J. Econ. Entomol.,* **50,** 698–99 (1957)
83. Izotova, T. E., Neklesova, I. D., Goryushin, V. A., and Kudrina, M. A., *Chem. Abstr.,* **52,** 4919 (1958)
84. Jameson, H. R., and Callan, I. W. M., *Nature,* **167,** 490 (1951)
85. Jameson, H. R., Thomas, F. J. D., and Tanner, C. C., *Ann. Appl. Biol.,* **38,** 121–34 (1951)
86. Jameson, H. R., Thomas, F. J. D., and Woodward, R. C., *Ann. Appl. Biol.,* **34,** 346–56 (1947)
87. Johannsen, O. A., and Patch, E. M., *Maine Agr. Expt. Sta. Bull. No. 195,* 229–48 (1911)
88. Johnson, D. R., *J. Econ. Entomol.,* **42,** 801–5 (1949)
89. Jones, D. P., *J. Sci. Food Agr.,* **7,** 562–65 (1956)
90. Jones, D. P. (Private communication, 1958)
91. Jones, E. W., Douglass, J. R., Parrish, C. P., and Jensen, V., *Proc. Am. Soc. Sugar Beet Technologists,* **7,** 490–96 (1952)
92. Jones, F. G. W., *British Sugar Beet Rev.,* **23**(3), 113–15 (1955)
93. Jones, F. G. W., and Humphries, K. P., *Ann. Appl. Biol.,* **41,** 562–77 (1954)
94. Kantack, E. J., *J. Econ. Entomol.,* **48,** 486 (1955)
95. Kantack, E. J., and Knutson, H., *J. Econ. Entomol.* (In press, 1958)
96. Kendrick, J. B., Jr., and Zentmyer, G. A., *Advances in Pest Control Research,* **1,** 219–75 (1957)
97. Kepner, R. A., and Leach,, L. D., *Agr. Eng.,* **30,** 519–23, 527 (1949)
98. King, K. M., Arnason, A. P., and Manson, G. F., *Dom. Can. Sci. Service Pub. No. 87,* 8 pp. (1948)
99. King, K. M., Forbes, A. R., Finlayson, D. G., Fulton, H. G., and Howitt, A. J., *J. Econ. Entomol.,* **48,** 470–73 (1955)
100. Kloor, L. A., Jr., *Proc. Am. Soc. Sugar Beet Technologists,* **6,** 61–69 (1950)
101. Kloor, L. A., Jr., *Bull. Calif. Beet Growers Assoc., Summary, 1951,* 35, 42 (1951)
102. Kloor, L. A., Jr. (Private communication, 1958)
103. Koehler, B., *Phytopathology,* **44,** 575–83 (1954)
104. Kramer, M., *Biológico,* **22,** 177–81 (1956)
105. Kulash, W. M., *J. Econ. Entomol.,* **46,** 433–41 (1953)
106. Kulash, W. M., *J. Econ. Entomol.,* **47,** 863–66 (1954)
107. Kulash, W. M., *Rev. Euclides,* **15,** 199–207 (1955)
108. Kulash, W. M., *J. Econ. Entomol.,* **49,** 65–67 (1956)
109. Kulash, W. M., and Munroe, R. J., *J. Econ. Entomol.,* **47,** 341–45 (1954)
110. Kulash, W. M., and Munroe, R. J., *J. Econ. Entomol.,* **48,** 11–19 (1955)

111. Lange, W. H., *Agr. Chem.*, **20**, 29–33, 68–69 (1947)
112. Lange, W. H. (Unpublished data,, Univ. of Calif., Davis, 1947–1958)
113. Lange, W. H., Carlson, E. C., and Corrin, W. R., *J. Econ. Entomol.*, **44**, 202–8 (1951)
114. Lange, W. H., Carlson, E. C., and Leach, L. D., *J. Econ. Entomol.*, **42**, 942–55 (1949)
115. Lange, W. H., Carlson, E. C., and Leach, L. D., *Calif. Agr.*, **7**, 7–8 (1953)
116. Lange, W. H., Lane, M. C., and Stone, M. W., *Proc. Intern. Congr. Entomol., 10th Meeting* (Montreal, Que., Canada, 1956, in press)
117. Lange, W. H., Leach, L. D., Kendrick, J. B., Jr., and Hills, F. J. (Processed publ., Univ. Calif. Davis, 10 pp., 1952)
118. Leach, L. D., Lange, W. H., Hills, F. J., and Kendrick, J. B., Jr., *Plant Disease Reptr.*, **38**, 193–99 (1954)
119. Leach, L. D., Lange, W. H., Weeks, W. F., and Hills, F. J. (Unpublished mimeo, Univ. of Calif., 7 pp., 1950)
120. Lilly, J. H., *Proc. Ann. Hybrid Corn Ind. Research Conf., 8th Meeting*, 76–92 (Chicago, Ill., 1953)
121. Lilly, J. H., *Proc. N. Central Branch Entomol. Soc. Am.*, **11**, 23–24 (1956)
122. Lilly, J. H., *Ann. Rev. Entomol.*, **1**, 203–22 (1956)
123. Lilly, J. H., and Gunderson, H., *Iowa Farm Sci.*, **9**(9), 9–11 (1955)
124. Long, W. H., *Iowa State College J. Sci.*, **32**(2), 213–15 (1957)
125. Long, W. H., and Lilly, J. H., *Bull. Entomol. Soc. Am.*, **2**, 27 (1956)
126. McEwen, F. L., Natti, J. J., Hervey, G. E. R., and Schroeder, W. T., *J. Econ. Entomol.*, **50**, 775–77 (1957)
127. McLean, D. M., *Phytopathology*, **47**, 557–59 (1957)
128. March, R. B., Metcalf, R. L., Fukuto, T. R., and Maxson, M. G., *J. Econ. Entomol.*, **48**, 355–63 (1955)
129. Masaitis, A. I., *Izvest. Sibir. Kraev. Stantz. Zashch. Rast.*, **2**(5), 53–65 (1927) [*Rev. Appl. Entomol.* **18**, 6 (1930)]
130. Masaitis, A. I., *Izvest. Sibir. Kraev. Stantz. Zashch. Rast.*, **3**(6), 1–41 (1929) [*Rev. Appl. Entomol.* **18**, 48 (1930)]
131. Matthewman, W. G., Dustan, A. G., and Davis, A. C., *Ann. Rept. Entomol. Soc. Ontario*, **72** (1941), 21–23 (1942)
132. Merkel, M. E., Pfrimmer, T. R., Furr, R. E., and Lloyd, E. P., *Bull. Entomol. Soc. Am.*, **3**, 45 (1957)
133. Merritt, J. M., *Agr. Food Chem.*, **6**, 184–87 (1958)
134. Metcalf, R. L., March, R. B., Fukuto, T. R., and Maxson, M. G., *J. Econ. Entomol.*, **48**, 364–69 (1955)
135. Miller, L. A., and Dustan, A. G., *Can. Dept. Agr. Sci. Serv. Pub. No. 912*, 4 pp. (1954)
136. Miller, L. A., and McClanahan, R. J., *Proc. Intern. Congr. Entomol., 10th Meeting* (Montreal, Que., Canada, 1956, in press)
137. Mitchell, W. C., *Iowa State College J. Sci.*, **31**(3), 480–81 (1957)
138. Morrison, H. E. (Private communication, 1957)
139. Mostafa, K. A., Newsom, L. D., Roussel, J. S., and Emerson, R. B., *J. Econ. Entomol.*, **47**, 684–91 (1954)
140. Natti, J. J., and Schroeder, W. T., *Plant Disease Reptr.*, **39**, 909–13 (1955)
141. Natti, J. J., Schroeder, W. T., Hervey, G. E. R., and McEwen, F. L., *Plant Disease Reptr.*, **42**, 127–33 (1958)

142. Nelson, W. L., Telford, H. S., and Harwood, R. F., *Wash. State Coll. Ext. Mimeo. No. 1644,* 3 pp. (1956)
143. Newlin, O. (Private communication, 1958)
144. Nugent, T. J., and Hofmaster, R. N., *Market Growers' J.,* **86,** 26–27 (1957)
145. Parencia, C. R., Jr., *Acco Press,* **35**(4), 2–4 (1955)
146. Parencia, C. R., Jr., Cowan, C. B., Jr., and Davis, J. W., *J. Econ. Entomol.* (In press, 1958)
147. Parencia, C. R., Jr., Davis, J. W., and Cowan, C. B., Jr., *J. Econ. Entomol.,* **50,** 31–36 (1957)
148. Parencia, C. R., Jr., Davis, J. W., and Cowan, C. B., Jr., *J. Econ. Entomol.,* **50,** 614–17 (1957)
149. Parkin, E. A., *Ann. Rev. Entomol.,* **1,** 223–40 (1956)
150. Popov, I. D., *Bulgar. Akad. Nauk. Inst. Biol. "Metodii Popov" Izvest.,* **7,** 111–28 (1956)
151. Potter, C., *Rothamsted Ann. Rept.,* 1955, 126–27 (1955)
152. Potter, C., Healy, M. J. R., and Raw, F., *Bull. Entomol. Research,* **46,** 913–23 (1956)
153. Rafaila, C., and Tusa, C., *Chem. Abstr.,* **52,** 3237 (1958)
154. Rawlins, W. A., *Bull. Entomol. Soc. Am.,* **3,** 40 (1957)
155. Rawlins, W. A., and Harris, E., Jr., *Farm Research,* July, 14 (1957)
156. Reed, J. R., *Proc. Assoc. South. Agr. Workers,* **53,** 127 (1956)
157. Reed, J. R., and Arant, C. H., *Proc. Assoc. South. Agr. Workers,* **54,** 146 (1957)
158. Reynolds, H. T., *Advances in Pest Control Research,* **2** (In press)
159. Reynolds, H. T., Anderson, L. D., and Andres, L. A., *J. Econ. Entomol.* (In press, 1958)
160. Reynolds, H. T., Anderson, L. D., and Swift, J. E., *J. Econ. Entomol.,* **46,** 555–60 (1953)
161. Reynolds, H. T., Fukuto, T. R., Metcalf, R. L., and March, R. B., *J. Econ. Entomol.,* **50,** 527–39 (1957)
162. Reynolds, H. T., Metcalf, R. L., and Winton, M., *Bull. Entomol. Soc. Am.,* **3,** 23 (1957)
163. Rhodes, A., *Grower,* **44**(26), 1510–11 (1955)
164. Ridpath, M. G., and Burton, R. K., *Agr. Rev. (London),* **2**(5), 39–42 (1956)
165. Ripper, W. E., *Advances in Pest Control Research,* **1,** 305–52 (1957)
166. Ripper, W. E., Greenslade, R. M., and Hartley, G. S., *Bull. Entomol. Research,* **46,** 481–501 (1950)
167. Ristich, S. S., and Schwardt, H. H., *J. Econ. Entomol.,* **42,** 77–80 (1949)
168. Robertson, R. L., *Proc. Assoc. Southern Agr. Workers,* **54,** 147 (1957)
169. Robertson, R. L., and Arant, F. S., *Proc. Assoc. Southern Agr. Workers,* **53,** 128 (1956)
170. Rudd, R. L., and Genelly, R. E., *Calif. Agr.,* **8,** 13 (1954)
171. Rudd, R. L., and Genelly, R. E., *Condor,* **57**(2), 117–18 (1955)
172. Rudd, R. L., and Genelly, R. E., *Calif. Dept. Fish Game, Game Bull. No. 7,* 209 pp. (1956)
173. Salkeld, E. H., and Scott, H. E., *J. Econ. Entomol.,* **51,** 165–68 (1958)
174. Schnathorst, W. C., *Phytopathology,* **44,** 588–92 (1954)
175. Srivastava, B. K., *Insecticides as seed protectants of the important cereals of Kansas* (Doctoral thesis, Kansas State Coll. of Agr. and Appl. Sci., Manhattan, Kansas, 78 pp., 1955)

176. Srivastava, B. K., and Bryson, H. R., *J. Econ. Entomol.*, **49**, 329–33 (1956)
177. Stanley, W. W., and Breeland, S. G., *Proc. Assoc. Southern Agr. Workers*, **54**, 147 (1957)
178. Starks, K. J., and Lilly, J. H., *J. Econ. Entomol.*, **48**, 549–55 (1955)
179. Starks, K. J., and Lilly, J. H., *J. Econ. Entomol.*, **48**, 538–43 (1955)
180. Starnes, O., and Filmer, R. S., *New Jersey Agr. Ext. Serv. Leaflet No. 78*, 3 pp. (1952)
181. Steudel, W., *Winter Congr. Intern. Research Sugar Beets, 21st Meeting* (Paris, France, 1958)
182. Stowe, B. B., and Yamaki, T., *Ann. Review Plant Physiol.*, **8**, 181–216 (1957)
183. Strong, F. E., *Proc. N. Central Branch Entomol. Soc. Am.*, **12**, 16–17 (1957)
184. Strong, F. E., *The Bionomics and Control of the Seed-Corn Maggot*, Hylemya cilicrura *(Rondani) on Field Crops* (Doctoral thesis, Univ. Wisconsin, Madison, Wis., 150 pp., 1958)
185. Tarr, S. A. J., *Ann. Appl. Biol.*, **41**, 578–85 (1954)
186. Thomas, C. A., *Penn. State Univ. Agr. Expt. Sta. Bull. No. 259*, 52 pp. (1930)
187. Thomas, C. A., *Penn. State Univ. Agr. Expt. Sta. Bull. No. 392*, 90 pp. (1940)
188. Toole, E. H., *U. S. Dept. Agr. Leaflet No. 220*, 8 pp. (1942)
189. Turner, N., *Conn. Agr. Expt. Sta. Bull. No. 501*, 95–96 (1945)
190. Van Dinther, J. B. M., *Rev. Appl. Entomol.*, [A]**43**, 33–34 (1955)
191. Walters, G. E., *Proc. Am. Soc., Sugar. Beet Technologists Proc.*, **8**, 372–75 (1954)
192. Ward, C. Y., *Proc. Assoc. Southern Agr. Workers*, **52**, 35 (1955)
193. Woodworth, C. E., *J. Agr. Research*, **57**, 229–38 (1938)
194. Wright, D. W., and Buxton, S. S., *Biol. Abstr.*, **52**, 3241 (1958)
195. Wright, J. M., *Ann. Appl. Biol.*, **44**, 561–66 (1956)

FLEAS AND DISEASE[1]

By William L. Jellison

U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratory, Hamilton, Montana

Introduction

In the broad field of disease caused by arthropod-borne agents there are many instances wherein the pathogenic organism appears to be well adapted to the vector and may even pass an essential part of its life cycle therein, as do the malaria parasites of man within the anopheline mosquitoes. The pathogen may be carried from stage to stage or even passed from one generation to another through the egg (transovarial passage). These adaptations of parasite to arthropod vector are thought to result from a long host-parasite association. The arthropod, in such instances, is conveniently referred to as a biologic vector of the pathogen.

In a few instances there is a closer relationship between vector and pathogen in which groups of related pathogens are transmitted by groups of related arthropods. Both pathogens and arthropods show about the same relative degree of diversity and homogeneity. Considerable host-parasite specificity is exhibited by both vector and parasite. An outstanding example of this degree of relationship is the relapsing-fever group of spirochetes of the genus *Borrelia* and their tick vectors of the genus *Ornithodoros*. Both are distributed in all the major faunal regions, in temperate and tropical zones, and occur in definite tick-spirochete combinations. These spirochetes exhibit a high degree of vector specificity, are maintained in part through transovarial passage, and develop in the body cavity of the tick. Only a single species is not carried by ticks, i.e., *Borrelia recurrentis* (Lebert), the cause of louse-borne relapsing fever of man.

The malarial parasites also fall in this category and are transmitted only by mosquitoes: human and other primate malaria by *Anopheles*, bird malaria by *Culex* and other genera. Transmission in nature is accomplished by no other means than the bite of mosquitoes.

The rickettsial diseases of man and animals are in general associated with ixodid ticks, but here there are more exceptions. The tick-borne rickettsiae are beautifully adapted to their vectors and are even maintained by transovarial passage, a phenomenon not well established for any pathogen of vertebrates, either virus, bacteria, or protozoan, carried by an insect.

There seems to be no better way to refer to an arthropod-pathogen combination within such groups than as an "evolutionary vector of the respec-

[1] The survey of the literature pertaining to this review was completed in June, 1958.

tive pathogen," meaning that vectors and pathogens evolved from an ancestral vector-pathogen combination. This interpretation is generally accepted for the parallelism that exists between vertebrates and some of their parasites, and it is especially well exemplified in the mammals and their ectoparasitic Anoplura, Mallophaga, and to some extent by their Siphonaptera (50, 121).

In regard to fleas, there are several instances in which they are well-adapted biologic vectors of a pathogen. The only indication of a role as "evolutionary vector" is in their relationship to the *Trypanosoma lewisi* group of trypanosomes. Some of these relationships will be mentioned in this introduction and discussed in greater detail in the individual disease sections.

Members of the genus *Pasteurella,* which includes the plague organism, *Pasteurella pestis* (Lehmann & Neumann), and the agent of tularemia, *Pasteurella tularensis* (McCoy & Chapin), constitute a group of bacteria highly pathogenic for birds and mammals. There is no indication that characteristic strains of *P. pestis* are related to any special species, genera, or families of fleas. While the organism is dependent upon fleas for its perpetuation and transmission, it does not invade the tissue of the flea, does not pass an essential part of its life cycle in the flea, and is frequently deleterious to the flea. There is much evidence that world-wide spread of plague has taken place within historic times. This would be interpreted as a poorly adapted biologic-vector relationship but not an evolutionary-vector relationship. The three other species of *Pasteurella* are not associated with insect transmission. Fleas have not been shown to be important vectors of tularemia, and the causative organism has definite biologic vectors, mainly among the ixodid ticks.

Murine typhus is the only rickettsial disease associated with fleas. Although most rickettsiae are carried by ticks and some by mites and lice, one (the agent of Q fever) may be quite independent of arthropod transmission. The organism of murine typhus is well adapted to flea transmission and multiplies intracellularly within the flea, where it is not noticeably harmful. There are no well-defined strains associated with certain genera of fleas, nor is there evidence to suggest the flea is more than a biologic vector.

Some mammalian trypanosomes are transmitted by tsetse flies, *Glossina,* and others by blood-sucking Hemiptera, *Triatoma* and related genera. However, there is the rather homogenous "*lewisi* group" that, with the exception of *Trypanosoma cruzi* Chagas, is carried by fleas. These pathogens parasitize a considerable variety of hosts in three orders, Rodentia, Insectivora, and Lagomorpha. The parasites invade flea cells, where they undergo a cyclic development, but they do not appear to be deleterious to the flea or to the natural mammalian hosts. Pathogen-vector-vertebrate associations are specific enough to suggest a long evolutionary relationship.

The relationship of fleas to plague, murine typhus, trypanosomiasis, and other diseases will be considered more thoroughly after a general discus-

sion of fleas themselves. We shall find that fleas probably are not the vectors of heart worm of dogs but may carry another dog filarid; that they are the alternate hosts for a dog tapeworm and two rodent tapeworms; that they may carry myxoma virus of rabbits, cause anemia, live in subcutaneous cysts, and inflict most grievous bites on man. It may be some consolation to learn that, in turn, fleas are parasitized by mites, nematodes, a chalcid fly, many protozoa, and are preyed upon by small beetles.

Fleas

In 1901, the Honorable N. Charles Rothschild, a banker of the House of Rothschild and a world authority on fleas, together with A. F. R. Wollaston, collected a number of fleas in Egypt and in the Sudan from various small mammals. At least five new species were represented in this collection. One of these was collected from *Acomys witherbyi* DeWinton, *Gerbillus robustus* (Cretzschmar), *Arvicanthis testicularis* Sundevall, *Dipodillus watersi* (DeWinton) *Dipus jaculus* (Linnaeus), and *Genetta dongolana* (Hemprich & Ehrenberg), all from Shendi. It was earlier collected from *Mus gentilis* Brants, near Suez, by Mr. W. E. DeWinton on October 17, 1900. This species was named *Pulex cheopis* when Rothschild described and figured it in 1903 (103). Little did these naturalists suspect that they were collecting and identifying one of the great insect panacides of all times, one which ranks with the yellow-fever mosquito, *Aedes aegypti* (Linnaeus), and the carrier of epidemic typhus, *Pediculus humanus* Linnaeus. The plague flea has also been known under the following names: *Pulex murinus* Tiraboschii, *Pulex philippinensis* Schultz & Herzog, *Xenopsylla pachyuromydis* Glinkiewicz, *Loemopsylla cheopis* Rothschild, and *Pulex tripolitanus* Fulmek. Today it is known as *Xenopsylla cheopis* (Rothschild).

Previous to the twentieth century, a rich literature on plague existed with contributions in poetry, fiction, history, and medical writings in several languages, a fine synopsis of which is given by Key's "The Plague in Literature" (62), with references dating from 430 B.C. to 1938. Fleas, on the contrary, do not have such a literary background prior to their definite association with plague about 1904.

In 1895, Baker (14) was able to list only 35 known species of fleas for the world, two of which had been described by Linnaeus in 1758. Baker assigned these to six genera and three families. By 1904 (15) he had catalogued 134 species and in the succeeding year added another 120 to the world list, many of these by his own descriptions. Holland (48) in a short synopsis of the history of Siphonaptera estimates there are now 1350 known species, divided among 200 genera.

The dramatic discovery that sylvatic plague was widespread in western United States in 1934 and 1935 and in Canada in 1939 was a great stimulus to flea studies in North America, and since that time many short papers and the following extensive and important works on fleas have appeared

in North American literature (32, 35, 47, 52, 56, 57, 110, 117). Prominent contributions to the world literature are papers of Uriarte (118), Costa Lima & Hathaway (66), Liu (69), Jameson (55), and Rosicky (101). The most ambitious venture in flea publications since the discontinuation of "Ectoparasites," [1915 to 1924 (60)] is the appearance of the *Catalogue of the Rothschild Collection of Fleas (Siphonaptera) in the British Museum,* which is edited by G. H. F. Hopkins & Miriam Rothschild (49). It is understood that eight volumes are planned for this catalogue, which is in reality a monograph of the fleas of the world. There are many publications on fleas, plague, and pertinent rodent ecology from the U.S.S.R. in both Russian and German literature [Ioff (53, 54)]. These are especially important to us because of the close taxonomic and ecologic relationship of Palearctic and Nearctic fauna, but this literature has not yet been integrated into our own studies.

Considerable literature exists on the origin of fleas and their affinity with other insects, much of which is referred to by Sharif (106). Agreement is lacking on these points but it can be said that the phylogeny of fleas has not been found in fossil records or in their own ontogeny. In parallel with other metazoan parasites, including insects, one would expect, except for the organs of reproduction, that evolution would be accompanied by a general simplification which would include reduction of the organs of locomotion (the wings are already obliterated except in the pupal stage) and reduction in ornamentation. In fleas, this would mean evolution from the large, ornate, nearly free-living, nest-inhabiting types, such as *Hystrichopsylla,* toward simplified, unornamented, fixed, cutaneous parasites with reduced thoracic segments, such as *Echidnophaga*; the evolution culminates, finally, in the fixed subcutaneous and almost spineless *Tunga.* In this view the writer is diametrically opposed to many competent workers, including Oudemans (90) and Sharif (106). Sharif states, ". . . this would tend to the conclusion that less hairy fleas without spines are more primitive." The subject is also discussed by Jordan (59). Proper orientation on this point would give much more meaning to flea taxonomy, which has already contributed much to the understanding of the biologic role of fleas in the transmission of disease agents.

Some attempts have been made to use fleas as an indicator of mammalian relationships. Related fleas are often found on related animals throughout the world. Most of the bat fleas belong to a single family whose members are not found on any other hosts. The rabbit fleas of Asia and of North America are related. The same is true of the ground-squirrel fleas. The thesis that flea phylogeny parallels host phylogeny has been discussed by Wagner (122) and has been more fully explored by Hopkins (51), who concludes ". . . the existing pattern is almost useless as a guide to the phylogeny of the hosts and almost never reliable for the chronology of the associations."

Plague

The plague bacillus is carried by fleas. . . . If this simple fact had been known in the twelfth century, the history of Europe and its colonies would have been different. What the difference would have been is difficult to imagine, but plague probably retarded western civilization by 200 years. Establishment of flea transmission depended upon development of the microscope, the beginning of a science of bacteriology, identification of the plague organism in man and rats, the formation of a hypothesis of plague transmission by fleas, and final experimental proof of the hypothesis. The discovery of the plague organism did not impose any special technical difficulty, but many other important pathogenic bacteria were discovered in a relatively short period of time just before the plague organism was described. The reason for this is that plague was not then present in Europe, the center of bacteriological science.

As the discovery of the role of fleas in the transmission of plague is inseparably linked to identification of the organism, it seems well to review that part of plague history in detail. The plague organism multiplied unseen, and perhaps unlooked for, in all the centuries of human history prior to 1894, and today it is not certain which of two men first discovered—within an interval of a few days—the etiological agent. The distinction must be assigned to either A. Yersin, a Dane, or S. Kitasato, a Japanese, or must be shared by them. Lagrange (64), who was at one time an assistant to Dr. Yersin in Indochina, has fairly presented the facts regarding the controversy over the discovery of the plague bacillus. The Hong Kong newspapers on June 14, 1894, announced that Kitasato had discovered an organism which he thought caused plague. His claim was challenged on the basis that some of his preparations were made from a corpse 11 hours after death, that his description did not fit the plague organism, and that he denied that his organism was the same as that described by Yersin. On June 20, Yersin wrote that he saw "very small rods, thick with rounded ends, and lightly colored (Löfflers blue)" in preparations from a bubo, and further ". . . my bacillus is probably that of plague but I am not certain." Yersin's description is consistent with characters of plague bacilli, and this description has enabled others to recognize the pathogens, insofar as is possible, from morphology alone.

Lagrange states, "In 1925, as chairman of the Congress of the Far Eastern Medical Association, before 400 members, amongst whom were 250 foreign delegates, Kitasato is to be honored for having publicly stated that Yersin alone was the discoverer of the plague bacillus." Both men had already established their status as bacteriologists of their period. Yersin had collaborated with Roux on the study of diphtheria and its toxin. Kitasato had cultured the bacillus of tetanus for the first time. The flames of nationalism have kept this controversy alive, although the principals long ago considered it settled. Perhaps we should give Kitasato more credit than

he gave himself in this moment of magnanimity and concede that he first saw the bacillus and that Yersin first described it accurately.

One of the first important developments following identification and culture of *Pasteurella pestis* by Yersin and by Kitasato was the final establishment that rat plague and human plague were caused by the same organism. The relation of rat epizootics to human epidemics had been observed since antiquity, the rat epizootic usually preceding the human cases.

Persistence and prevalence of plague in India after its subsidence in many other countries invited, if not demanded, action from the scientific world, and in 1904 steps were taken in England to organize an advisory committee and a working commission, later known as the "India Plague Commission," to inquire into the problem. Results of this inquiry appear in the literature as *Reports on Plague Investigations in India,* usually without identification of specific scientists. The work of the Commission was so important that it seems fitting to name the early members, who were: Charles Martin, George Lamb, William Glen Liston, George Ford Petrie, Sydney Rowland, Thomas Henry Gloster, M. Kasava Pai, V. L. Manker, P. S. Ramachandrier, and C. R. Arvi. No doubt personnel of the Commission changed as work progressed.

A historical review on insect transmission of plague was prepared by the Advisory Committee of the India Plague Commission and published (1) as an introduction to the reports of their own experimental work. The Committee gave generous credit to many investigators for contributions leading to the conclusion that the plague bacillus is transmitted by fleas. Yersin (131), Hankin (43), and Nuttall (87) found virulent plague bacilli in dejecta of flies and ants that fed on infected organs. Nuttall fed bugs (presumably bed bugs) on infected mice and found that they harbored the bacilli but did not transmit them by bite. Ogata (88) injected crushed fleas from rats dead of plague into two mice, one of which died of plague after three days. He suggested, from epidemiological considerations, that plague was conveyed mostly by suctorial insects such as mosquitoes and fleas.

Simond (109) found organisms morphologically indistinguishable from plague bacilli in the stomach of fleas which had fed upon rats and mice dying of plague, and he succeeded in infecting a mouse by injecting an extract of crushed fleas taken from a plague rat. He found that in the absence of fleas plague was not transmitted from sick or dead rats to healthy rats in close proximity, but in at least two instances he observed transmission when fleas were present. He conjectured (wrongly) that the actual mode of transmission was by contamination of the skin with infected flea feces at the site of bite.

On purely epidemiological grounds based on observations of plague in Sidney, Australia, Thompson (116) arrived at a "theory of plague" that involved or necessitated transmission by fleas. Gauthier & Raybaud (39) repeated Simond's experiments and were able to transmit infection by fleas from rat to rat at least five times. Probably at least some of their fleas

were *Xenopsylla cheopis* (Rothschild). Liston (68) found that guinea pigs exposed in plague houses in Bombay became infested with rat fleas and then died of plague.

Other contemporary investigators in the early part of the century experienced conflicting results and some discounted the role of fleas as vectors of plague. Their results and views are more understandable now that the taxonomy and biology of fleas are better known, both because some fleas have proved to be very inefficient vectors, or show little tendency to bite man, and because of susceptibility of some hosts and resistance of others to plague infection.

The early work of the Commission (1) seemed well oriented by the findings of Yersin and Kitasato and by the experimental results of Simond and of Gauthier & Raybaud. The ingenuity, emphasized by simplicity, of their experiments led to the following results and conclusions, which with some refinements have been universally accepted. (*a*) "The presumption that plague was transferred from sick to healthy rats by the agency of fleas." This was based on 30 positive transfers out of 50 completed attempts. (*b*) "The possibility of the rat flea, *X. cheopis,* carrying plague from one rat to another is therefore demonstrated directly." This was based on 21 positive transfers out of 38 completed experiments in which normal animals had no other exposure to plague than fleas from known infected hosts.

After these basic issues were settled, the India Plague Commission advanced to other studies, including anatomy of the rat flea and "the mechanism by which the flea infects a healthy animal" (2). The workers determined that plague bacilli multiply in the stomach of a flea and that infection could result from the bite of a single flea. They could not find bacilli in body cavity or salivary glands and concluded, "No evidence has been obtained in favor of infection by contaminated mouth parts or regurgitation from the stomach, but the possibility of infection by such means cannot be excluded." They had studied the internal anatomy of fleas and were familiar with the function of the proventriculus. They followed development and multiplication of plague bacilli in the flea stomach and seriously considered the possibility of infection by regurgitation. It seems to this writer that they should have been rewarded by the ultimate discovery of how plague is transmitted by fleas.

The fine point of infection by regurgitation remained obscure until 1914, when it was elucidated at the Lister Institute in England by Bacot & Martin (11), and presented in a brief but historically important paper from which I quote:

> In a proportion of infected fleas the development of the bacilli was found to take place to such an extent as to occlude the alimentary canal at the entrance to the stomach. The culture of pest appears to start in the intercellular recesses of the proventriculus, and grows so abundantly as to choke this organ and extend into the oesophagus. Fleas in this condition are not prevented from sucking blood as the pump is in the pharynx, but they only succeed in distending an already contami-

nated oesophagus, and, on the cessation of the pumping act, some of the blood is forced back into the wound. Such fleas are persistent in their endeavours to feed, and this renders them particularly dangerous. Fleas suffering from obstruction do not necessarily perish, and in the course of some days the culture obliterating the lumen of the proventriculus may autolyse and passage again become pervious.

This discovery did much to clarify the role of fleas in the transmission of plague and to harmonize the conflicting results of many previous experiments and experimenters.

After plague appeared in Hong Kong and Canton in 1894, it spread rapidly to other coastal cities of the world. In 1899, there were at least three instances of plague on ships entering United States ports (67). It is not likely that plague became established in our ports from any of these ships. However, on March 6, 1900, plague was diagnosed in a Chinese resident in San Francisco. Sporadic cases occurred there throughout the summer and fall, and diagnosis of these cases stirred up a controversy that swept through newspapers, political and medical circles, and even into the courts, which attempted to establish by judicial pronouncement that plague was not present in San Francisco. The story is well told by Kellogg (61) and should be read in its entirety. Some measure of the emotions aroused are indicated in his statement:

> They [newspapers] launched a campaign of vilification against the Health Board and the Federal Quarantine Officer, Dr. Kinyoun, that for unexampled bitterness, unfair and dishonest methods, probably never had been and never again will be equalled.

At the request of Surgeon J. H. White of the United States Marine Hospital Service (forerunner of the United States Public Health Service), the Secretary of Treasury, L. J. Gage, appointed a committee of prominent bacteriologists to settle the question. This committee consisted of Professors Simon Flexner of the University of Pennsylvania, F. G. Novy of the University of Michigan, and L. F. Barker of the University of Chicago. In spite of the efforts of the governor of California, some members of the legislature and the president of the University of California to block their work, they found plague present in San Francisco. The toll of plague in this first epidemic for San Francisco, ending in 1904, was recorded as 121 cases and 118 deaths.

Plague reappeared in San Francisco in 1907 and occurred in Seattle the same year, in New Orleans in 1912, in other Gulf Coast cities in 1920, and in Los Angeles in 1924. Vigorous rat- and flea-control measures have eliminated it from our cities insofar as can be determined, but plague still persists in wild rodent populations (sylvatic plague).

The more serious aspects of sylvatic plague are a matter of record. The disastrous pneumonic plague epidemics that ravaged Manchuria in 1910 and 1911 and in 1920 and 1921 started among hunters and trappers who were taking marmots, *Marmota bobac* Pallas. The toll of the former epidemic was estimated at 60,000 and of the latter at 9300 victims. Since then the suspi-

cion has been entertained that sylvatic infection has a greater tendency to induce pneumonia than rat plague and therefore to become highly contagious (79).

The discovery in 1908, by the United States Public Health Service, that plague in North America was no longer confined to rats and rat fleas but had become established in ground squirrels, *Citellus beecheyi* (Richardson) in Contra Costa County, California, was an incentive for a new line of study on taxonomy, biology, disease, and control of both native rodents and their flea parasites. (An unexpected development from this study was the discovery of a new disease, tularemia, in Tulare County, California, where it was first known as a "plague-like disease of rodents.") This work was further stimulated when plague was found in native rodents in Modoc County of northern California [1934], then in Oregon [1935], in Montana east of the Continental Divide [1935], and in Alberta, Canada [1939]. A systematic survey of rodents and rodent fleas for plague which was already in progress was greatly intensified and its range extended. Most of the state health departments in the West cooperated with the United States Public Health Service Laboratory in San Francisco in this survey and plague was found to be endemic in 14 western states and two Canadian provinces, Alberta and Saskatchewan. It was found in 38 species and subspecies of rodents and lagomorphs. The Sciuridae or squirrel family were most prominent carriers [Meyer (78)]. Over 4000 isolations of plague were made [Link (67)]. This type of plague has come to be known as sylvatic or campestral plague and is present in many parts of the world.

As to the future of plague, I should like to quote from Pollitzer (96):

> However, even though plague, which but a few decades ago ranked high among the diseases decimating mankind, now occupies a rather inconspicuous place in the fatality lists, it would be wrong to assume that this infection has altogether lost its sting.

The last chapter on plague has not been written, but a measure of its present status is given in *Time* (9), which states:

> The World Health Organization announced in Geneva that in 1957 only 514 deaths due to plague were reported in the free world and only 44 of them in India. At long last, it looked as though the Black Death was licked.

The work of the India Plague Commission, the Manchurian Plague Commission, the microbiologists and entomologists in U.S.S.R. and in the U. S. Public Health Service, and of thousands of unnamed rat catchers has contributed to this achievement.

Practical world-wide control of the unholy trinity, *Rattus rattus, Xenopsylla cheopis,* and *Pasteurella pestis* is a *fait accompli.*

Tularemia

Pasteurella tularensis (McCoy & Chapin), the etiologic agent of tularemia, has many vertebrate reservoirs and arthropod vectors in nature and

other potential vectors that have been implicated by laboratory experiment only. Ticks are especially efficient vectors and often carry the bacterium to man. In some ticks the organism passes transovarially as well as from larva to nymph to adult. Deer flies of one species only, *Chrysops discalis* Williston, are important carriers of infection to man in the western United States.

The first scientific paper on tularemia [McCoy (73)] reported recovery of infection from fleas, *Diamanus montanus* (Baker) (=*Ceratophyllus acutus* Baker), taken from a sick or dead ground squirrel and tested in guinea pigs. McCoy attempted to transmit the disease by placing healthy animals in cages with flea-infested sick squirrels. In several experiments, transmission was effected, but in one experiment presence of buboes in the cervical region suggested transfer by ingestion rather than by flea bite.

Transmission of *P. tularensis* from sick to healthy water rats by fleas, *Megabothris walkeri* (Rothschild), is recorded by Olsufiev (89), who also reported: recovery of infection from *Ctenophthalmus assimilis* (Taschenberg) and *Ctenophthalmus pollex* Wagner & Ioff in nature; persistence of infection for four months in *Neopsylla setosa* Wagner; and laboratory transmission with *C. assimilis, Ctenophthalmus agyrtes* (Heller), *Amphipsylla rossica* Wagner, and *Ctenopsylla segnis* (Schönherr). He did not consider fleas efficient vectors.

In one report of the Minnesota Wildlife Disease Investigation, Green, Evans, Bell & Larson (41) recorded the recovery of *P. tularensis* from one lot of four fleas removed from a snowshoe rabbit, *Lepus americanus* Erxleben, and from three lots of one, nine, and three fleas, respectively, from cottontail rabbits, *Sylvilagus floridanus* (Allen). All were tested by animal inoculation. In no instance was infection recovered from fleas when it was not demonstrated in the host, either snowshoe hare or cottontail. The fleas concerned in these tests are referred to as "*Spilopsyllus cuniculi* (Dale)," which is the European rabbit flea, whereas the common fleas on snowshoe hares are *Hoplopsyllus glacialis lynx* (Baker) and the characteristic fleas on cottontails in Minnesota are *Cediopsylla simplex* (Baker) and *Odontopsyllus multispinosus* (Baker). Waller (123) later recovered *P. tularensis* from fleas, *C. simplex,* taken from a sick cottontail rabbit in Iowa.

Tularemia infection is very infrequently recovered when wild-rodent fleas are tested for plague at the San Francisco Laboratory. These findings are usually announced in the Public Health Reports without authorship. One such report deals with recovery of infection from fleas of prairie dogs, *Cynomys leucurus leucurus* Merriam, collected in Wyoming (5) and another with fleas from ground squirrels in Alberta, Canada (6).

Prince & McMahon (99) found that *P. tularensis* persisted as long as 32 days in *Xenopsylla cheopis* (Rothschild) although neither this species nor *Diamanus montanus* (Baker) transmitted infection by bite. They also found that rabbit fleas (*Cediopsylla*) became infected with tularemia, but their transmission experiments were limited and unsuccessful.

There is very little or nothing in the epidemiology of tularemia in man to

suggest that fleas are important vectors (37). However, human infection is not a good indicator of the role of fleas as vectors among wild rodents because wild-rodent fleas do not have the intimate contact with man or the predilection to bite him that rat fleas have. It is possible that fleas are more important in the spread of tularemia in rabbits and rodents than is now recognized, and the subject invites further investigation.

Salmonellosis

The transmission of *Salmonella enteritidis* (Gaertner) by *Pulex irritans* (Linnaeus) and *Ctenocephalides canis* (Curtis) was studied by Varela & Olarte (119). They found that the pathogens survived in fleas up to 96 hours, but transmission by bite was not demonstrated.

In the course of laboratory investigations on plague, Eskey, Prince & Fuller (31) found that some of their fleas had become accidentally infected with *S. enteritidis*. They then demonstrated that *X. cheopis* and *Nosopsyllus fasciatus* (Bosc) could transmit the infection to mice. The exact mode of transmission was not determined, but regurgitation into the bite wound seemed probable. Large numbers of organisms were found in flea feces, and in some instances excessive defecation indicated that the infection was deleterious to the flea. The same flea species were also infected with *Salmonella typhimurium* (Loeffler) but did not transmit infection to mice.

Murine Typhus

Fleas are generally accepted to be the vectors of murine typhus, also known as endemic typhus, flea-borne typhus, Mexican typhus (in part), and tabardillo. The name "Brill's disease" has been applied to this entity but in a strict sense it is not appropriate; the reasons will be discussed later. The causative organism of flea-borne typhus is *Rickettsia (Rickettsia) typhi* (Wolbach & Todd). For a discussion of the nomenclature of this organism the reader is referred to Mooser (81) and Philip (92).

The evidence that fleas are the only vector or even the principal vector of the rickettsia of murine typhus is not conclusive, and the exact method of transmission has not been established. Transmission may be by flea or mite bite, by passage of infection through the skin where it has been abraded by scratching flea bites and contaminated by infected flea feces, by inhalation or ingestion of rickettsia-laden flea feces or rickettsia-laden rodent urine in the dust of buildings. All methods may be effective at one time or another.

The significant steps in the identification of murine typhus as a disease entity, distinct from epidemic typhus, and studies on probable arthropod vectors of murine typhus are deserving of review. Dyer (27) states that epidemic typhus was brought to Canada in 1659 and that over 20,000 deaths from typhus occurred among Irish immigrants in Canada about 1847. It is accepted that epidemic louse-borne typhus was prevalent in eastern North America at one time but probably died out before 1900. Brill (18) recognized a typhuslike disease which he could not accept as epidemic typhus and

in a publication which is a classic in medical science recorded 221 cases. Most of the cases were in New York City. This entity or complex of diseases has been designated correctly as "Brill's disease," but its full significance was not realized until careful epidemiologic and laboratory work identified at least three distinct kinds of typhuslike disease in the Eastern Seaboard states, namely, recrudescent epidemic typhus, flea-borne typhus, and spotted fever. Two other rickettsial diseases, rickettsialpox and Q fever, are present in the area, but have little or no clinical resemblance to the typhus fevers.

Although many writers have referred to the murine or flea-borne typhus of the southeastern states as "Brill's disease," on the basis of presently known geographic distribution it is quite likely that Brill in New York City was dealing exclusively with recrudescent epidemic typhus, and, therefore, the name "Brill's disease" is not applicable to murine typhus. "Brill's disease," which we considered to be a distinct entity, was shown by Zinsser (132) and Plotz, Wertman & Bennett (93) to be a long-delayed recrudescence of Old World typhus, usually in immigrants, caused by the organism *Rickettsia prowazeki* da Rocha Lima, and mainly confined to the large eastern cities.

Another typhuslike disease was identified in the Eastern Coastal states in 1932 when Badger (12) isolated the rickettsia of spotted fever, *Rickettsia* (*Dermacentroxenus*) *rickettsii* (Wolbach, 1919) from ticks, *Dermacentor variabilis* (Say). Spotted fever has since been found widespread with many important endemic foci in the eastern states. Certainly, prior to 1932, some cases of spotted fever were diagnosed as "Brill's disease" or murine typhus.

Paullin (91) in Georgia, where murine typhus is now known to be common, was among the first to recognize a clinical typhus fever without mortality, thus distinct from classical typhus. Neill (84) observed that certain strains of Mexican typhus produced orchitis in a large proportion of experimental male guinea pigs and that swelling was suggestive of spotted fever rather than European typhus. Mooser (80) confirmed this finding and interpreted it as a "biological difference" between the two types of typhus. Much work was done on typhus fevers in Mexico, but in published reports it is difficult or impossible to tell just when workers were dealing with flea-borne typhus and when with epidemic typhus. Both forms were present and both were referred to as tabardillo or Mexican typhus.

After a careful epidemiologic study, Maxcy (77) postulated the necessity of a rodent reservoir and insect vector for typhus in the eastern states. It remained for Dyer, Rumreich & Badger (29) to make the first isolation of murine typhus from rat fleas collected at a typhus focus in Baltimore. Dyer, Ceder, Rumreich & Badger (28) later showed that the organisms of murine typhus persisted in rat fleas for at least nine days and were present in feces of infected fleas. They were also successful in experimental transmission of murine typhus from rat to rat with *X. cheopis*.

Studies on multiplication of the rickettsia in fleas were done by Dyer, Workman, Ceder, Badger & Rumreich (30) and by Mooser & Castañeda (82).

The former authors found that in rat fleas, *X. cheopis,* fed on infected animals the agent passed an incubation period of two or three days and the fleas became highly infectious on the fifth or sixth day. In three instances, an inoculum representing "1/128,000th of a flea" produced infection. This was the highest dilution tested. Fleas remained infectious for 40 days and presumably for life without evidence of ill effect. Mooser & Castañeda (82) followed, by cytologic methods, the development and multiplication of the rickettsia of murine typhus in tissues of several species of fleas, including *X. cheopis, Nosopsyllus fasciatus* (Bosc), *Leptopsylla musculi* (Duges), *Ctenocephalides canis* (Curtis), *and Ctenocephalides felis* (Bouche). They found that the rickettsia multiplied abundantly in epithelial cells of the stomach, but that the organisms were prevented by the peritrophic membrane from entering the lumen of the gut in quantity. Multiplication also took place in cells of the malpighian tubules and these cells were probably the source of organisms found in the lumen of the gut and feces. They considered fleas to be relatively inefficient vectors of typhus.

Early workers on murine typhus were disappointed in the vector efficiency of fleas, for they found that when experimental hosts were carefully protected from contamination with flea feces, infection was not transmitted by feeding alone. However, when feces from infected fleas were rubbed into abraded skin, as would occur when flea bites were scratched, infection resulted [Ceder, Dyer, Rumreich & Badger (20)].

Serious doubt as to the exclusive role of fleas in transmission of endemic typhus was first introduced by Dove & Shelmire (24, 25), following their laboratory studies with the tropical rat mite, *Ornithonyssus bacoti* (Hirst) (=*Liponyssus bacoti*). They were able to transmit the disease from guinea pig to guinea pig and from guinea pig to rat with these mites. They demonstrated transovarial passage of the organism in mites. This cosmopolitan mite frequently bites man and is sometimes abundant. Contemporary critics [see (24) for abstracts of discussion] expressed some skepticism that Dove & Shelmire were actually working with a strain of endemic typhus, but this criticism has not persisted.

Mooser, Castañeda & Zinsser (83) found that the rat louse was readily infected with murine typhus and transmitted the disease from rat to rat under simulated natural conditions. While it may be important, as they suggest, in maintaining enzootic infection, this louse does not bite man and would not be a direct cause of human infection. In order to clarify the rather confused vector relationships in endemic typhus, research workers then turned to extensive ecologic and epidemologic studies. Such studies have been reported by Rumreich & Koepke (104) for Florida, Alabama, and Honolulu and by Fox (36) for Puerto Rico. Their reports indicate that a tick and mites may be of some importance as vectors but they do not challenge seriously the theory that fleas are the principal carriers to man.

A more extensive review of arthropods as vectors of endemic typhus was prepared by Kohls (63). It is now accepted that rat fleas of several species

play a prominent part as vectors of this disease. For a very extensive list of references on murine typhus, the reader is referred to *Bibliography on Epidemic, Endemic, and Scrub Typhus Fever* (7).

From about 1913, when "endemic typhus" was first recognized in the southern states, to 1945 there was a rapid rise in the number of cases reported for each consecutive five-year period, increasing from 199 in 1916 to 1920 to 21,572 in 1941 to 1945 [Andrews & Link (4)]. No doubt most of the increase may be attributed to "heightened awareness of the disease, improved diagnostic facilities, and more adequate case reporting on the part of attending physicians," as Andrews & Link state. The marked increase from 1936 to 1940 (11,299 cases), when the disease had become well known, to 1941 to 1945 (21,572 cases) strongly suggested increased prevalence in old areas and extension of infection into new localities. This rapid rise in case incidence prompted a vigorous campaign of research and control by the several states and the Public Health Service. The recorded case incidence reached a peak in 1944 with 5401 cases. In 1945 the incidence dropped slightly to 5193 cases and then rapidly declined for seven consecutive years to 186 cases for 1952, the last year for which figures are given by Pratt & Good (98). No doubt many of the measures applied in this widespread murine-typhus control program contributed to this decline, but Pratt (97) and Pratt & Good (98) give much credit to the general use of DDT dust, both as a rodenticide and as an insecticide.

Dyer (26) found three kinds of native wild rodents in the eastern United States susceptible to infection with endemic typhus. These were the woodchuck, *Marmota monax monax* (Linnaeus); the meadow mouse, *Microtus pennsylvanicus pennsylvanicus* (Ord); and the white-footed mouse, *Peromyscus leucopus noveborascensis* (Fischer). The introduced house mouse, *Mus musculus* Linnaeus, was also found to be susceptible. Indigenous rodents do not seem to be important as reservoirs of endemic typhus, in contrast to rats and mice of the introduced family Muridae in North America.

Woodward (128) states that, as a result of nearly world-wide search for disease agents stimulated by World War II, murine typhus is now known to be prevalent in North Africa, the West Indies, South America, the Philippines, and in all European and Asiatic countries.

Ioff (54) mentions a typhus of spermophiles, genus *Citellus,* in Russia that may be distinct from the well-known murine typhus. If this is the case, it is an extremely interesting discovery.

As facts from nature, the hospital, and the laboratory become known regarding a widespread disease, its epidemiology increases in complexity until it is no longer safe to make any positive, unqualified statement about it. Perhaps the best we can say in summary is that endemic typhus is a distinct entity but closely related to, and possibly derived from or progenitor to, classical epidemic typhus (13). It is primarily a disease of murine rodents, which include only the introduced rats, genus *Rattus,* and mice, *M. musculus,* in North America. It is spread from rodent to rodent by their fleas, lice, and possibly mites, and occasionally to man from rats and mice, presumably by

fleas. The most likely mode of infection is by organisms penetrating abraded skin at the site of flea bites contaminated by flea feces. Infection by direct bite remains a possibility, and infection by inhalation and ingestion is probable.

Murine typhus has practically disappeared from many large cities and has experienced a dramatic drop in incidence throughout the United States coincidental with vigorous control measures based on the premises outlined above.

Myxomatosis

Myxomatosis is a virus disease of wild and domestic rabbits and was first found in a native rabbit, *Sylvilagus braziliensis* (Linnaeus), in Brazil. It is transmitted by a great variety of blood-sucking insects but mosquitoes appear to be the most effective vectors (120). Aragão (10) and Day (22) studied transmission with *Ctenocephalides felis* (Bouche), which was able to transmit the virus but was not as efficient as three species of mosquitoes used in one experiment (22). Ratcliffe (100) stated:

> Circumstantial evidence has suggested transmission by the cat flea in one outbreak in northern New South Wales in 1952; but against this we have to record the failure of the infection to spread in rabbit populations infested with stick-fast fleas (*Echidnophaga* sp.) in parts of western Australia, despite large-scale inoculation.

Bull & Mules (19) also worked with a stickfast flea, *Echidnophaga myrmecobii* Rothschild, and found it was not an efficient vector. Day (22) states, "It is regrettable that no work has been published on the mechanism of transmission by the rabbit flea, *Spilopsyllus,* which many English workers consider has been mainly responsible for the spread of the disease in Britain."

This deficiency has been in part corrected by Lockley (70) who succeeded in transmitting myxoma virus in seven of 10 attempts with the European rabbit flea, *Spilopsyllus cuniculi* (Dale).

It seems logical that some of the true rabbit fleas, *Spilopsyllus, Hoplopsyllus, Cediopsyllus,* or *Odontopsyllus,* would be more efficient vectors of a rabbit disease than *Echidnophaga,* which is not a specific rabbit parasite and has the added disadvantage of restricted mobility.

Myxomatosis has been introduced with considerable success into Australia for control of the European rabbit. It has also caused extensive and much publicized epidemics in wild rabbits in Europe and Great Britain since 1953. Under natural conditions the virus does not appear to spread to animals other than lagomorphs.

Trypanosomiasis

Fleas are vectors of certain trypanosomes, including *Trypanosoma lewisi* (Kent), of small mammals. This particular group of trypanosomes is considered to be nonpathogenic, in contrast to the virulent African species. Taliaferro (115) says that rodent trypanosomes ". . . are morphologically

identical or similar to *T. lewisi* of the rat, and are differentiated almost entirely by their specificity for their rodent hosts." A good account of *T. lewisi* and related forms is given by Wenyon (126). Hoare (46) places *Trypanosoma cruzi* in the "*lewisi* group." *T. cruzi* is the cause of Chagas's disease but is carried by *Triatoma* and related genera of Hemiptera. *T. lewisi* has been a classical organism for laboratory study and demonstration because of the ease with which it is maintained. It has been useful in the study of trypanosome life history and in immunology and therapy of trypanosomiasis.

T. lewisi multiplies in both mammalian host and flea vector. Organisms in the blood are taken up by the flea and may multiply in the lumen of the gut. However, some penetrate and multiply within the cells lining the stomach. Organisms re-enter the stomach by rupture of cells and are passed in flea feces. Organisms in various stages of development are found in fresh feces and are infectious when ingested by rats. Ingestion of infected fleas would be equally infectious. There is general agreement that this is the usual mode of rat infection, although one worker, Yamasaki (130), claimed that the dog flea can transmit trypanosomes by its proboscis. Transmission by other ectoparasites, including the rat louse, has been studied.

Insofar as other species of this group have been tested, they have been found to be transmissible by fleas, for example, *Trypanosoma duttoni* Thiroux of the mouse by a bird flea *Ceratophyllus hirudinis* Curtis; *Trypanosoma rabinowitschi* Brumpt of the hamster by *Typhlopsyllus assimilis* and *N. fasciatus;* and *Trypanosoma nabiasi* Ralliet of the rabbit by *Spilopsyllus cuniculi.*

The life cycle of the rabbit trypanosome, *Trypanosoma nabiasi,* in the rabbit and in the flea, *S. cuniculi,* has been determined by Grewal (42) who found that the parasite multiplies in the spleen of the rabbit and in the gut of the flea.

Several species of trypanosomes are present in some indigenous North American rodents, lagomorphs, and insectivores outside the known range of any *Triatoma* or related genera, and we may assume that they are transmitted by fleas. These *Trypanosoma* include *T. leporis-sylvaticus* Watson, *T. peromysci* Watson, *T. citelli* Watson, *T. evotomys* Hadwen, and *T. soricis* Hadwen of Canada (125), and *T. parkeri* Dias (23) from the marmot in Montana. *T. neotomae* Wood is present in wood rats, *Neotoma* spp., and in wood-rat fleas in California (127). The flea vector was given by Wood (127) as *Orchopeas wickhami* (Baker), which is a tree-squirrel flea, but it is more likely one of the many subspecies of *Orchopeas sexdentatus* (Baker) which are characteristic wood-rat parasites.

With the exception of *T. cruzi,* a species transmitted by Hemiptera, none of the rodent trypanosomes is known to be pathogenic for man. Fleas may be suspected as vectors of trypanosomes of birds in the northern part of our continent, although Herman (44) thinks that mosquitoes may be carriers. Bishopp (16) stated that the dog flea and human flea were suspected of car-

rying leishmaniasis in the Mediterranean region but other arthropods, *Phlebotomus,* have now been identified with this disease.

Some parasitic flagellates of fleas are occasionally mistaken for developing stages of mammalian trypanosomes.

Only in the trypanosomes do we have a group of related organisms transmitted by fleas. This host-parasite-vector association suggests a long evolutionary relationship.

Filariasis and Other Nematode Infestations[2]

Both mosquitoes and fleas have been considered as possible vectors of heart worm of dogs, *Dirofilaria immitis* (Leidy), because developmental stages have been found in them. In Australia, Breinl (17) found filarial larvae which he thought were *D. immitis* in fleas, *Ctenocephalides canis* and *Ctenocephalides felis.* Summers (114), in New Orleans, found many infected fleas, *C. canis, C. felis,* and *Pulex irritans,* on dogs and concluded: "It appears both biologically and epidemiologically fleas are more suitable intermediate hosts of *D. immitis* than had been previously supposed."

The role of fleas in relation to *D. immitis* transmission has recently been questioned by Newton & Wright (86) whose work "may shed light on some of the apparent discrepancies and unexplained findings reported for the dog heart worm." They have determined the existence of at least two types of microfilariae common in North American dogs. One is associated with adult heart worms, *D. immitis,* and develops in mosquitoes, *Anopheles quadrimaculatus* Say. In later experiments, Newton (85) reported the transmission of *D. immitis* by bite of *A. quadrimaculatus.* The other, probably the larva of *Dipetalonema reconditum* (Grassi), which is a parasite in subcutaneous tissues, develops in fleas, *C. canis* and *C. felis,* but fails to develop in mosquitoes. Successful transmission experiments with *D. reconditum* have not been reported.

In taxonomic flea studies, the writer has occasionally noted nematodes within the bodies of rodent fleas. Alicata (3) reported spirurid larvae in *C. felis* in Nebraska and cited a reference to spirurid larvae in *X. cheopis* and *N. fasciatus* in Australia. Sassuchin, Ioff & Tiflow (105) figure an adult nematode, *Neonema ctenophthalmi,* from *Ctenophthalmus pollex* and list other records of parasitism of fleas by nematodes.

Cestode Infestations

The cysticercoid stages of several tapeworms develop in fleas. One is the common cat and dog parasite, *Dipylidium caninum* (Linnaeus). Its immature stages have been found in *C. canis, C. felis, and P. irritans.* The life

[2] Since the preparation of this manuscript, the writer has received a relevant study: L. Kartman, "The Vector of Canine Filariasis; A Review With Special Reference to Factors Influencing Susceptibility," *Rev. brasil. malariol. e doenças trop.,* **8**(5), 1–41 (1957)

cycle was partly determined by Grassi & Rovelli (40). The mouth parts of an adult flea are not adapted to ingest a large tapeworm egg and this part of the cycle remained in doubt until Joyeux (58) showed that fleas become infected as larvae and that the cysticercoids remain viable and infective in the adult flea after metamorphosis. *D. caninum* is of medical importance, as it occasionally infests children. A closely related species, *Dipylidium sexcoronatum* von Ratz, develops cysticercoids in the biting louse of dogs, *Trichodectes canis* DeGeer. Stewart (111) has shown that physiological differences in respect to chemotherapy also exist between the two species, whose confusion has no doubt caused much difficulty in earlier life-cycle studies.

Some authors would greatly reduce the number of species in the genus *Dipylidium,* but Wardle & McLeod (124) list 20 species and provide a key to 13 species which were recognized by Lopez-Neyra (71). The life cycles of most of these have not been determined, but one may suspect that fleas are involved as hosts to other species besides *D. caninum.*

Wardle & McLeod (124) also list the following fleas as intermediate hosts of rodent tapeworms; *X. cheopis, C. canis,* and *P. irritans* for *Hymenolepis nana* Siebold; *Nosopsyllus fasciatus* (Bosc), and *X. cheopis* for *Hymenolepis diminuta* Rudolphi. These two cestodes have many other insect hosts, especially beetles. Although they are essentially rodent parasites, they frequently infest children.

Anemia

An anemia caused by excessive numbers of fleas, *C. canis,* on fox pups on a fur farm is described by Law & Kennedy (65). Red-blood-cell counts as low as 2,600,000 were observed after an infestation period of 15 days, whereas the normal count was about 7 million. When the fleas were removed, the animals recovered and the normal count was soon established. The anemia was attributed entirely to exsanguination by the fleas.

Anemia and even rapidly fatal exsanguination are often observed when rats or other small experimental animals are introduced into a vigorous colony of fleas.

Dermatitis

Some idea of the importance of fleas as pests in the San Francisco Bay area is given by Lunsford (72) who in a paper entitled "Flea Problem in California" quotes the miserable experiences related by early travelers and newcomers. One of the more gifted men of literature wrote of his encounter with fleas, "If any sinning soul ever suffered the punishment of purgatory . . . those torments were endured by myself that night." Some new arrivals were consoled by the prediction that ". . . they would get used to the fleas in time"; thus they were offered a layman's concept of immunity to flea bites, a concept that is now generally accepted. The pest potential of fleas was also admitted by residents of other areas who participated in the dis-

cussion of Lunsford's paper. This writer can only comment that a knowledge of some of the finer points of flea taxonomy in no way mitigates the misery of a dozen or two bites inflicted by Yunnan rat fleas.

The production of desensitizing antigens for flea-bite victims was started in 1939 at the University of California and at the Hooper Foundation for Medical Research. Cherney, Wheeler & Reed (21) desensitized susceptible people with antigens made from fleas collected from dogs. McIvor & Cherney (74, 75) later reared quantities of fleas on laboratory animals for antigen production. Flea antigens were used with "encouraging results" on 128 hypersusceptible people. Follow-up on 82 patients elicited the following responses: 16 reported fewer bites after treatment; 17 stated their reactions were less severe; 43 replied that their bites were not only less severe but fewer in number; five stated they were not benefited; and one child was reported to have aggravated reactions to flea bites following the injections. A major project for study of sensitivity to flea bites is again in progress at the Kaiser Foundation in co-operation with the United States Public Health Service Laboratory in San Francisco.

Our work in public health must be guided by principles such as those expressed, respectively, by the surgeon general of the Public Health Service and by the World Health Organization Constitution (8): "Public health has become more than the absence of disease"; "Health is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity." Within these guide lines the pest flea and the pest mosquito must go, along with the plague flea and the malaria mosquito. Presumably the pest-flea problem has been ameliorated by DDT and other modern insecticides, but the writer finds no documentary evidence of this. Flea-bite censuses have not been customary.

Tunga Infestations

There is a special pathological condition, caused by fleas of the genus *Tunga*. This insect is also known as the jigger or chigoe. Jiggers are often confused with "chiggers," which are larval trombiculid mites and quite a different pest. Hopkins & Rothschild (49) recognize six species of *Tunga*, of which only one, *Tunga penetrans* (Linnaeus) is a human parasite.

After fertilization, the female *Tunga* penetrates or firmly attaches to the skin of its host—bird, man, or other mammal—usually on the feet. It is said that the flea burrows, but it is poorly equipped for such action. Somehow the skin envelops the flea except for a small sinus with an external aperture through which eggs and dejecta are passed. This attachment causes intense itching and frequent ulceration as the flea grows to about the size of a small pea. This flea is known in Africa, South America, and adjacent islands.

There is one record of larvae of *Tunga* infesting skin lesions [Faust & Maxwell (33)]. The presence of flea larvae, *Hoplopsyllus glacialis glacialis* (Taschenberg), in the soiled and matted fur of the arctic hare is recorded

by Freeman & Madsen (38). This appears to be normal for the species but is a most unusual habit for flea larvae.

Parasites and Predators of Fleas

One of the first reports of adult fleas parasitized with mites was by Fox (34) who found three rat fleas, *Nosopsyllus fasciatus* (Bosc), so infested. Such mites are observed by nearly everyone who examines large numbers of fleas. Some of the mites, at least, are the hypopial stage of Tyroglyphidae and are merely riders. Some are attached completely exteriorly, and some are found beneath overlapping sclerites. While the mites are not considered to be injurious, a flea so parasitized is often conspicuous for its rough appearance, displaced sclerites, and debris under sclerites. Infestations of 1 to 10 mites per flea are observed and the writer has found them most commonly on rodent fleas of the genus *Opisocrostis*. Sassuchin, Ioff & Tiflow (105) figure another type of mite of the genus *Uropoda* which attaches to fleas by a long posterior stalk, again obviously a rider.

Rothschild & Clay (102), in the popular book *Fleas, Flukes and Cuckoos,* illustrate the mites that infest fleas and credit Leeuwenhoek with the observation that mites prey on larvae of the pigeon flea. They state that this fact inspired the oft-quoted lines by Jonathan Swift, "Big fleas have little fleas upon their back to bite 'em and little fleas have lesser fleas and so ad infinitum." Rothschild & Clay also cite two instances of phoresy in which mallophaga were attached to fleas.

A hymenopterous parasite of tree-squirrel-flea larvae, *Orchopeas wickhami,* in England is reported by Sikes (107) as *Bairamlia fuscipes* Waterston. Numerous small beetles are known to be predatory on fleas [Sassuchin, Ioff & Tiflow (105)].

Miscellaneous Diseases and Microorganisms Associated With Fleas

In addition to the more familiar pathogens which are transmitted by fleas as discussed previously, Steinhaus (113) lists a number of microorganisms that have been associated with fleas in nature or by experiment. Although some of these are potent human pathogens, e.g., the leprosy and glanders bacilli, subsequent experience has failed to show that their flea association is of any epidemiological significance. Others listed are interesting organisms of possible significance to fleas but of no direct importance to human disease. The flea species and their respective microbial associates are as follows:

Ceratophyllus columbae (Walckenaer & Gervais): *Legerella parva* Nöller
Ceratophyllus gallinae (Schrank): *Legerella parva* Nöller
Ceratophyllus sp.: *Herpetomonas pattoni* Swingle
Ctenocephalides canis (Curtis): *Herpetomonas ctenocephali* Mackinnon; *Nosema ctenocephali* Kudo; *Nosema pulicis* Nöller; *Mycobacterium*

leprae (G. A. Hansen) [the leprosy bacillus]; Unidentified organisms

Ctenocephalalides felis (Bouché); *Rickettsia burnetii* Derrick (the organism of Q fever); *Spirochaeta ctenocephali* Patton

Ctenophthalmus agyrtes (Heller): *Crithidia ctenophthalmi* (Mackinnon)

Hystrichopsylla talpae (Curtis): Unidentified "symbiotes"

Leptopsylla segnis (Schönherr): *Herpetomonas ctenopsyllae* Laveran & Franchini

Monopsyllus sciurorum (Schrank) (= *"Ceratophyllus sciurorum"*): *Herpetomonas debreuli* Brumpt

Nosopsyllus fasciatus (Bosc); *Agrippina bona* Strickland; *Legerella grassi* Splendore; Unidentified "symbiotes"

Pulex irritans (Linnaeus): *Diplococcus pneumoniae* Weichselbaum (pneumonia bacillus); *Leptomonas pulicis* Patton & Rao; *Mycobacterium leprae* (G. A. Hansen); *Salmonella choleraesuis* (Smith); Unidentified organisms

Pulex sp.: *Herpetomonas pattoni* Swingle

Xenopsylla cheopis (Rothschild): *Malleomyces pseudomallei* (Whitmore)

Xenopsyllus cleopatrae (Rothschild): *Crithidia cleopatrae*

Flea larvae: *Pseudomoas aeruginosa* (Schroeter); *Salmonella enteritidis* (Gaertner); *Staphylococcus albus* Rosenbach; *Staphylococcus aureus* Rosenbach

In "Materials for the Study of the Parasites and Enemies of Fleas," Sassuchin, Ioff & Tiflow (105) mention many of the microorganisms listed by Steinhaus and, in addition, include: *Malpigiella refringens* Minchin; *Actinocephalus parvus* Wellmer; *Steina rotundata* Ashworth & Rettie; *Gregarina ctenocephalus* Ross.

The role of these organisms as etiologic agents of disease of vertebrates is either unimportant or unknown.

In an extensive paper in Russian on fleas and disease, Ioff (54) gives a table listing 25 diseases associated with fleas. Some of these which have not been discussed in this review are pasteurellosis of chickens, pneumococcus of rodents, staphylococcus of hares, leprosy of rats, bartonellosis of dogs, and anthrax.

Addendum

In the preparation of this review, emphasis has been placed on the earlier, historic aspects of the subject which it seemed useful to assemble with proper citations under one title. Many seemingly trivial observations and references have been cited either because they illustrate some interesting biological principle of disease transmission by fleas or because they would be overlooked in a casual survey of the literature. A publication by Simmons & Hayes (108), "Fleas and Disease," has been of much help.

The serious student of plague cannot miss the important summary papers by Wu (129), Pollitzer (94, 95, 96), Hirst (45), Macchiavello (76), and Swellengrebel (112).

Knowledge gained in the study of fleas and disease has soon been applied to practical control, and, although our information on some of these diseases is still incomplete, the diseases have been controlled. For example, the student of human plague would be hard pressed to find clinical material in this or any country. Endemic and epidemic areas on distribution maps are shrinking. Disease incidence is decreasing. These are rewards each public-health worker can share, no matter how insignificant his own contribution may be.

LITERATURE CITED

1. Advisory Committee, *J. Hyg.*, **6**, 425–34 (1906)
2. Advisory Committee, *J. Hyg.*, **7**, 395–420 (1907)
3. Alicata, J. E., *J. Parasitol.*, **21**, 221–22 (1935)
4. Andrews, J. M., and Link, V. B., *Pests*, **15**, 12–20 (1947)
5. Anonymous, *Public Health Repts. (U. S.)*, **56**, 1521 (1941)
6. Anonymous, *Public Health Repts. (U.S.)*, **57**, 1358 (1942)
7. Anonymous, *Bibliography on Epidemic, Endemic, and Scrub Typhus Fevers* (Technical Library, Camp Detrick, Frederick, Md., 63 pp., 1952)
8. Anonymous, *Public Health Repts. (U. S.)*, **72**, 842–46 (1957)
9. Anonymous, *Time*, **71** (7), 45 (Feb. 17, 1958)
10. Aragão, H. de B., *Brasil-méd.*, **33**, 74 (1920)
11. Bacot, A. W., and Martin, C. J., *J. Hyg.*, Plague Supplement III, 423–439 (1914)
12. Badger, L. F., *Public Health Repts. (U. S.)*, **47**, 2365–69 (1932)
13. Baker, A. C., *Am. J. Trop. Med.*, **23**, 559–66 (1943)
14. Baker, C. F., *Can. Entomologist*, **27**, 221–22 (1895)
15. Baker, C. F., *Proc. U. S. Natl. Museum*, **27**, 365–469 (1904)
16. Bishopp, F. C., *U. S. Dept. Agr. Bull. No. 248*, 1–31 (1915)
17. Breinl, A., *Ann. Trop. Med. Parasitol.*, **14**, 389–92 (1921)
18. Brill, N. E., *J. Med. Sci.*, **139**, 484–502 (1910)
19. Bull, L. B., and Mules, M. W., *J. Council Sci. Ind. Research*, **17**, 79–93 (1944)
20. Ceder, E. T., Dyer, R. E., Rumreich, A., and Badger, L. F., *Public Health Repts. (U. S.)*, **46**, 3101 (1931)
21. Cherney, L. S., Wheeler, C. M., and Reed, A. C., *Am. J. Trop. Med.*, **19**, 327–32 (1939)
22. Day, M. F., *J. Australian Inst. Agr. Sci.*, **21**, 145–51 (1955)
23. Dias, E., *Trans. Roy. Soc. Trop. Med. Hyg.*, **31**, 260 (1936)
24. Dove, W. E., and Shelmire, B., *J. Am. Med. Assoc.*, **97**, 1506–11 (1931)
25. Dove, W. E., and Shelmire, B., *J. Parasitol.*, **18**, 159–68 (1932)
26. Dyer, R. E., *Public Health Repts. (U. S.)*, **49**, 723–24 (1934)
27. Dyer, R. E., *J. Am. Med. Assoc.*, **124**, 1165–72 (1944)
28. Dyer, R. E., Ceder, E. T., Rumreich, A., and Badger, L. F., *Public Health Repts. (U. S.)*, **46**, 2415–16 (1931)
29. Dyer, R. E., Rumreich, A., and Badger, L. F., *Public Health Repts. (U. S.)*, **46**, 334–38 (1931)
30. Dyer, R. E., Workman, W. G., Ceder, E. T., Badger, L. F., and Rumreich, A., *Public Health Repts. (U. S.)*, **47**, 987–94 (1932)
31. Eskey, C. R., Prince, F. M., and Fuller, F. B., *Public Health Repts. (U. S.)*, **64**, 933–41 (1949)
32. Ewing, H. E., and Fox, I., *U. S. Dept. Agr. Misc. Publ., No. 500*, 142 pp. (1943)
33. Faust, E. C., and Maxwell, T. A., *Arch. Dermatol. and Syphilol.*, **22**, 94–97 (1930)
34. Fox, C., *Entomol. News*, **20**, 216 (1909)
35. Fox, I., *Fleas of Eastern United States* (Iowa State College Press, Ames, Iowa, 191 pp., 1940)
36. Fox, I., *J. Parasitol.*, **37**, 85–95 (1951)

37. Francis, E., *Public Health Repts. (U. S.)*, **52,** 103–13 (1937)
38. Freeman, R. B., and Madsen, H., *Nature,* **164,** 187–188 (1950)
39. Gauthier, J. C., and Raybaud, A., *Compt. rend. soc. biol.,* **54,** 1497 (1902)
40. Grassi, G. B., and Rovelli, G., *Centr. Bakteriol. Parasitenk.,* **5,** 370–77 (1889)
41. Green, R. G., Evans, C. A., Bell, J. F., and Larson, C. L., *Role of Fleas in the Natural Transmission of Tularemia* (Minnesota Wildlife Disease Investigation, Mimeographed Report), 25–28 (April, 1938)
42. Grewal, M. S., *Parasitology,* **47,** 100–18 (1957)
43. Hankin, E. H., *Ann. inst. Pasteur,* **12,** 705 (1898)
44. Herman, C. M., *Bird-Banding,* **15,** 89–112 (1944)
45. Hirst, L. F., *The Conquest of Plague* (Oxford University Press, London, England, 478 pp., 1953)
46. Hoare, C. A., *Parasitology,* **28,** 98–109 (1936)
47. Holland, G. P., *Can. Dept. Agr. Tech. Bull. No. 70,* 306 pp. (1949)
48. Holland, G. P., *Can. Dept. Agr. Sci. Serv.,* 585–88 (1953)
49. Hopkins, G. H. E., and Rothschild, M., *An Illustrated Catalogue of the Rothschild Collection of Fleas* (Cambridge University Press, Cambridge, England, I, 361 pp., II, 445 pp., 1953 and 1956)
50. Hopkins, G. H. E., *Proc. Zool. Soc. London,* **119,** 387–604 (1949)
51. Hopkins, G. H. E., *First Symposium on Host Specificity Among Parasites of Vertebrates,* 64–87 (University of Neuchâtel, Neuchâtel, Switzerland, 324 pp., 1957)
52. Hubbard, C. A., *Fleas of Western North America* (Iowa State College Press, Ames, Iowa, 533 pp., 1947)
53. Ioff, I. G., *Z. Parasitenk.,* **9,** 72–134 (1936)
54. Ioff, I. G., *Questions of the Ecology of Fleas in Connection With Their Epidemiological Significance* (Ordzonikidze Regional Anti-Plague Station, Pyatigorsk, U.S.S.R., 116 pp., 1941)
55. Jameson, E. W., Jr., *FEC Pamphlet 8–2* (Bull. Office Chief Surgeon, U. S. Army Forces Far East, 21 pp., 1953)
56. Jellison, W. L., and Good, N. E., *Natl. Inst. Health Bull. No. 178,* 193 pp. (1942)
57. Jellison, W. L., Locker, B., and Bacon, R., *J. Parasitol.,* **39,** 610–18 (1953)
58. Joyeux, C., *Bull. soc. pathol. exotique,* **9,** 578–83 (1916)
59. Jordan, K., *Overgedr. Tijdschr. Entomol.,* **88,** 79–83 (1947)
60. Jordan, K., and Rothschild, N. C., *Ectoparasites I* (Hazell, Watson, and Viney, Ltd., London and Aylesbury, England, Parts 1–6, 380 pp., 1915–1924)
61. Kellogg, W. H., *Am. J. Public Health,* **10,** 835–44 (1920)
62. Keys, T. E., *Bull. Med. Library Assoc.,* **32,** 35–56 (1944)
63. Kohls, G. M., "Vectors of Rickettsial Diseases," in *Rickettsial Diseases of Man,* 83–96 (American Association for Advancement of Science, Washington, D. C., 247 pp., 1948)
64. Lagrange, E., *J. Trop. Med. Hyg.,* **29,** 299–303 (1926)
65. Law, R. G., and Kennedy, A. H., *Ann. Rept. Ontario Government Exptl. Fur Farm* (1933)
66. Lima, A. da Costa, and Hathaway, C. R., *Pulgas: Bibliografia, Catalogo e Hospedores (Monografias Inst. Oswaldo Cruz No. 4)* 522 pp. (1946)
67. Link, V. B., *Public Health Monograph No. 26,* 120 pp. (1955)
68. Liston, W. G., *J. Bombay Nat. Hist. Soc.,* **16,** 253–72 (1905)

69. Liu, C. Y., *Philippine J. Sci.*, **70**, 1–122 (1939)
70. Lockley, R. M., *Vet. Record*, **66**, 434–35 (1954)
71. Lopez-Neyra, C. R., *Bull. soc. pathol. exotique.*, **21**, 239–53 (1928)
72. Lunsford, C. J., *Arch. Dermatol. and Syphilol.*, **60**, 1184–1202 (1949)
73. McCoy, G. W., *Public Health Bull. No. 43*, 53–71 (1911)
74. McIvor, B. C., and Cherney, L. S., *Am. J. Trop. Med.*, **21**, 493–97 (1941)
75. McIvor, B. C., and Cherney, L. S., *Am. J. Trop. Med.*, **23**, 377–79 (1943)
76. Macchiavello, A., *J. Trop. Med.*, **57**, 3–8, 45–48, 65–69, 87–94, 116–21, 139–46, 158–71, 191–97, 220–24, 238–43, 275–79, 294–98 (1954)
77. Maxcy, K. F., *Public Health Repts. (U. S.)*, **41**, 2967–95 (1926)
78. Meyer, K. F., *Ann. N. Y. Acad. Sci.*, **48**, 429–67 (1947)
79. Meyer, K. F., *Public Health Repts. (U. S.)*, **72**, 705–19 (1957)
80. Mooser, H., *J. Infectious Diseases*, **43**, 241–61 (1928)
81. Mooser, H., *Am. J. Trop. Med.*, **28**, 841–43 (1948)
82. Mooser, H., and Castañeda, M. R., *J. Exptl. Med.*, **55**, 307–23 (1932)
83. Mooser, H., Castañeda, M. R., and Zinsser, H., *J. Exptl. Med.*, **54**, 567–75 (1931
84. Neill, M. H., *Public Health Repts. (U. S.)*, **32**, 1105–8 (1917)
85. Newton, W. L., *J. Parasitol.*, **43**, 589 (1957)
86. Newton, W. L., and Wright, W. H., *J. Parasitol.*, **42**, 246–58 (1956)
87. Nuttall, G. H. F., *Centr. Bakteriol. Parasitenk.*, **22**, 97 (1897)
88. Ogata, M., *Centr. Bakteriol. Parasitenk.*, **21**, 769–77 (1897)
89. Olsufiev, H. G., *Parasitology of Tularemia* (Khatenever, L. M., Ed., Union Institute of Experimental Medicine, Moscow, U.S.S.R., 211 pp., 1943)
90. Oudemans, A. C., *Novitates Zool.*, **16**, 133–58 (1909)
91. Paullin, J. E., *Southern Med. J.*, **6**, 36 (1913)
92. Philip, C. B., *Ann. N. Y. Acad. Sci.*, **56**, 484–94 (1953)
93. Plotz, H., Wertman, K., and Bennett, B. L., *The Serological Pattern in Epidemic Typhus Fever*. (Report to the Director, U. S. A. Typhus Commission, 1944)
94. Pollitzer, R., *Bull. World Health Organization*, **4**, 475–533 (1951)
95. Pollitzer, R., *Bull. World Health Organization*, **2**, 337–76 (1952)
96. Pollitzer, R., *Bull. World Health Organization*, **9**, 131–70 (1953)
97. Pratt, H. D., *Ann. N. Y. Acad. Sci.* (In press, 1958)
98. Pratt, H. D., and Good, N. E., *J. Parasitol.*, **40**, 113–29 (1954)
99. Prince, F. M., and McMahon, M. C., *Public Health Repts. (U. S.)*, **61**, 79–85 (1946)
100. Ratcliffe, F. N., *J. Australian Inst. Agr. Sci.*, **21**, 130–33 (1955)
101. Rosicky, B., *Fleas of Czechoslovakia* (Československé Akademie Ved., Prague, Czechoslovakia, 439 pp., 1957)
102. Rothschild, M., and Clay, T., *Fleas, Flukes, and Cuckoos* (Collins, St. James Place, London, England, 304 pp., 1952)
103. Rothschild, N. C., *Entomologist's Monthly Mag.*, **39**, 83–87 (1903)
104. Rumreich, A. S., and Koepke, J. A., *Public Health Repts. (U. S.)*, **60**, 1421-28 (1945)
105. Sassuchin, D., Ioff, I., and Tiflow, W., *Rev. microbiol. epidémiol. parasitol.*, **15**, 27–44 (1936)
106. Sharif, M., *Proc. Pakistan Sci. Conf., 4th Meeting*, 1–35 (Peshawar, Pakistan, 1952)

107. Sikes, E. K., *Parasitology*, **22**, 361–69 (1930)
108. Simmons, S. W., and Hayes, W. J., *Proc. Intern. Cong. Trop. Med. Malaria, 4th Meeting*, Paper No. 12, 1678–89 (Washington, D.C., 1948)
109. Simond, P. L., *Ann. inst. Pasteur*, **12**, 625–87 (1898)
110. Snodgrass, R. E., *Smithsonian Misc. Collections*, **104**(18), 1–89 (1946)
111. Stewart, M. A., *J. Parasitol.*, **25**, 185–86 (1939)
112. Swellengrebel, N. H., *J. Hyg.*, **48**, 135–45 (1950)
113. Steinhaus, E. A., *Insect Microbiology* (Comstock Publishing Co., Inc., Ithaca, N. Y., 763 pp., 1946)
114. Summers, W. A., *Proc. Soc. Exptl. Biol. Med.*, **43**, 448–450 (1940)
115. Taliaferro, W. H., "Nonlethal Infection with the *Trypanosoma lewisi* Group of trypanosomes," in *Protozoa in Biological Research* (Columbia University Press, New York, N. Y., 1148 pp., 1941)
116. Thompson, J. A., *J. Hyg.*, **6**, 537–69 (1906)
117. Traub, R., *Fieldiana: Zool. Memoirs*, **1**, 1–124 (1950)
118. Uriarte, L., *Rev. inst. bacteriol.*, **6**, 57–98 (1934)
119. Varela, G., and Olarte, J., *Science*, **104**, 104–5 (1946)
120. Various Authors, *J. Australian Inst. Agr. Sci.*, **21**, 130–51, 250–53 (1955)
121. Various Authors, *First Symposium on Host Specificity Among Parasites of Vertebrates* (University of Neuchâtel, Neuchâtel, Switzerland, 324 pp., 1957)
122. Wagner, J., *Zoogeographica*, **1**, 263–68 (1932)
123. Waller, E. F., *Vet. Student*, **2**, 54, 55, 73 (1940)
124. Wardle, R. A., and McLeod, J. A., *The Zoology of Tapeworms* (University of Minnesota Press, Minneapolis, Minn., 780 pp., 1952)
125. Watson, E. A., and Hadwen, S., *Parasitology*, **5**, 21–26 (1912)
126. Wenyon, C. M., *Protozoology: A Manual for Medical Men, Veterinarians and Zoologists*, **1** (William Wood and Co., New York, N. Y., 778 pp., 1926)
127. Wood, F. D., *Univ. Calif. (Berkeley) Publ. Zoöl.*, **41**, 133–44 (1936)
128. Woodward, T. E., "Endemic (Murine) Typhus Fever: Symptomatology," in *Rickettsial Diseases of Man*, 134–38 (American Association for Advancement of Science, Washington, D. C., 247 pp., 1948)
129. Wu, Lien-Teh, in *Plague: A Manual for Medical and Public Health Workers*, Chap. 1 (National Quarantine Service, Shanghi Station, 547 pp., 1936)
130. Yamasaki, S., *Arch. Protistenk.*, **48**, 137 (1924)
131. Yersin, A., *Ann. inst. Pasteur*, **8**, 666 (1894)
132. Zinsser, H., "Epidemiology and Immunity in the Rickettsial Diseases," in *Virus and Rickettsial Diseases*, 872–907 (Harvard University Press, Cambridge, Mass., 907 pp., 1940)

INSECTS AND THE EPIDEMIOLOGY OF MALARIA

By Paul F. Russell
The Rockefeller Foundation, New York; Malaria Consultant, World Health Organization

Definition

As the derivation of the word suggests (*epi*, upon; *demos*, the people), "epidemiology" may be defined as the sum of what is known about disease in communities rather than in individuals. Thus, the epidemiology of human malaria is concerned with the various factors that determine the frequencies and distributions of the disease as it invades, inhabits, and leaves groups of people. Russell (110) has narrated the history of malaria epidemiology; Boyd (9), Macdonald (79), and Russell *et al.* (111) have described the present status of this subject in full detail.

Insects influence the epidemiology of malaria in man only through a single genus, *Anopheles*, of the family Culicidae in the order Diptera. Most of the approximately 370 species within this genus are not closely enough associated with man to be important, but in the females of more than 60, the four species of plasmodia of human malaria may find definitive hosts and vectors.

Basic Principles

Plasmodium falciparum (Welch), *Plasmodium malariae* (Laveran), *Plasmodium ovale* (Stephens), and *Plasmodium vivax* (Grassi & Feletti) all develop asexually in the erythrocytes of man. From 11 to 30 days after infection, depending on species and strain of plasmodium, sexual gametocytes of the parasite appear in the peripheral blood. Their development goes no farther in man. But if male and female gametocytes are taken into the gut of a susceptible mosquito, mating takes place, followed by oöcyst development on the gut wall of the insect. When ripe, the oöcyst liberates sporozoites, most of which lodge in the mosquito's salivary ducts and glands. Thereafter, the insect injects infective sporozoites into its host whenever it feeds. Thus, excluding accidental infections by blood transfusion or hypodermic injection, the spread of malaria may be described as a chain of infection from man to anopheline to man.

But there are a number of modifying conditions that influence this basic chain. Indeed, endemic and epidemic malaria depend on the interplay of many factors, as illustrated in simple form by the following formula (111) which includes the principal variables of which malaria is a function:

$$(X + Y + Z)\ \text{bepti} = \text{Malaria Incidence}$$

The chain of malarial infection:

X = the human carrier
Y = the mosquito vector
Z = the human victim

modified by:

b = bionomics of man and mosquito
e = environment of man and mosquito
p = plasmodia—species and strains
t = therapy and control measures
i = immune factors in man and mosquito

From the time of Hippocrates to the middle of the nineteenth century, the epidemiology of malaria was largely concerned with circumstances and surroundings that seemed to produce intermittent fevers, for example, with marshes and with comets. This approach might be termed *circumstantial epidemiology* (79). Then came the discoveries of the malaria plasmodium in 1880 and of its mosquito vector, in 1897 and 1898. Naturally, epidemiological studies were thereafter evermore strictly devoted to the bionomics of parasite, mosquito, and man, and *biological epidemiology* (79) developed. But, as Macdonald (79) has pointed out, biological data about malaria—even when thoroughly investigated and quite well known, as in West Africa and Ceylon—were not adequate to explain some striking differences in the behavior of the disease. Why, for instance, was the disease fickle in Ceylon, liable to explode into devastating epidemics, but remarkably stable in West Africa, where malaria epidemics occurred only on the fringes of the malaria zones? Obviously, epidemiological data were not adequate, although in each area the bionomics of the vector mosquitoes were much studied. Most lacking has been a sense of proportion. To meet this need there is now being developed a third type of study, namely, *mathematical epidemiology* (79).

Mathematical Epidemiology

Previous mathematical studies of malaria by Ross (107, 108), Waite (128), and especially by Lotka (69) were thorough and yet were disappointing in their failure, for example, to explain such puzzles in malaria epidemiology as stable and unstable malaria, mentioned above. Believing that although the mathematical approach has heretofore been largely sterile it yet is a logical one, Macdonald (79) has in recent years re-examined the question and has succeeded in integrating the mathematical analysis of the interaction of numerical factors with the facts found in the field. He discovered a fundamental mistake in the premises of previous analyses, namely the assumption that superinfection could not occur. Undoubtedly, an individual infected with malaria can be reinfected before complete recovery from his initial infection. Macdonald has developed a mathematical model with superinfection as one of the premises and has succeeded in building up a new and most useful account of the epidemiology of malaria (71 to 79).

The chief elements that go into the formation of Macdonald's mathe-

matical model are the following: (*a*) anopheline density in relation to man; (*b*) average number of persons bitten by one mosquito during one day; (*c*) proportion of anophelines with sporozoites in their salivary glands; (*d*) proportion of these which is actually infective; (*e*) probability that vector anopheline will survive one whole day; (*f*) days required for completing the extrinsic cycle under local conditions; (*g*) proportion of the population bitten by mosquitoes during one day; (*h*) proportion showing parasitemia; (*i*) proportion of (*h*) who have received a single infective inoculum and who revert to the uninfected state in one day; (*j*) the "incubation interval," which is the average time in days from the ingestion of gametocytes by an anopheline to the appearance of infective gametocytes in a second case infected by it; (*k*) the "reproduction rate," or the number of secondary infections originating from a single primary case; (*l*) infant parasite rates and gametocyte rates and counts, classified into three-month age groups.

The chance of an anopheline's becoming a malaria vector varies to some extent according to species and strain of plasmodia, but unless a species of *Anopheles* is almost totally refractory, the variations in susceptibility are relatively minor factors, in contrast to, for example, the importance of man-biting habits, food preferences, and average life expectancy of the insect vector.

Once implanted in a susceptible insect, the plasmodium develops through the oöcyst stage to the infective or sporozoite stage in from 8 to 35 days or more, depending on species of plasmodium and on the external temperature. For example, sporozoites of *Plasmodium vivax* may develop in 8 days at 28°C. to 30°C. or in 16 to 17 days at 20°C.; *P. malariae* in 25 to 28 days at 23°C. to 24°C. or in 30 to 35 days at 20°C.; *P. falciparum* in 10 to 12 days at 26°C. to 27°C. or 22 to 23 days at 20°C.; *P. ovale* in 16 days at 25°C. Development does not occur below 19°C. in *P. falciparum* and 15°C. in *P. vivax*, and it is uncertain in both species at temperatures above 32°C. (9, 111).

The infectiousness of the insect to man is related to the density of the infection in the mosquito. If there are over 50 oöcysts present, on the average, in a batch of infected mosquitoes, these insects will be infectious to about 50 per cent of the persons fed upon. Anophelines with fewer oöcysts infect lesser percentages of their hosts.

The frequency of feeding in anophelines seems to depend on the rate of ovarian development, which in turn is greater at higher temperatures. But within the range of temperatures normal in malarious areas, feeding commonly occurs every other day. The species vary greatly in choice of host: some, like *Anopheles fluviatilis* James, are anthropophilic; some like *Anopheles litoralis* King, are zoophilic; and some, like *Anopheles minimus flavirostris* (Ludlow), are attracted to man or animals according, apparently, to their proximity to one or the other. Food preferences are investigated by precipitin tests which, for purposes of the epidemiology of malaria, need

only to separate the source of blood meals into two classes—man and animal. Standard tests are given by Boyd (9) and Russell *et al.* (111); newer developments and theory by Weitz (130, 131).

The expectation of life of an anopheline species is an important factor in the spread of malaria. Most adult mosquitoes seem to have a high death rate for a day or two after emergence, then a relatively long period of two or three months or more when they are not so susceptible to death from natural causes, and finally a terminal stage when death is caused by old age. However, during the middle period, hazards of natural enemies and climate are great so that most anophelines are not long-lived. Obviously, after sporozoites have lodged in the salivary ducts, the spread of infection will be governed by the length of life of the insect and the frequency with which it feeds on man. Too little attention has been paid to the measurement of these factors in the field. However, studies by Davidson & Draper (21) and by Davidson (20) have given useful information on this subject and point the way to the type of malaria survey now required.

Macdonald (79) explains the important concept of a *reproduction rate* as follows:

> One may imagine an individual suffering from falciparum infection who is infective to all the mosquitoes which feed on him during 80 days (or half of those which feed on him for 160). If 10 bite each day he originally infects 800 mosquitoes. These might have a probability of survival through one day of 0.9; if the temperature were such that the extrinsic cycle lasted 12 days, 28 per cent of these would survive to the development of sporozoites and they would have a subsequent expectation of life of 10 days. If the mosquito were entirely anthropophilic, feeding once every 2 days on man, the survivors would on the average each convey the infection to 5 people. Through this mechanism 1,120 infections might be distributed in the population from the primary case. In the immediate context it is no matter that a number of these infections would be nullified by overlapping on the same individual. Obviously nothing approaching such a rate of multiplication could go on unchecked for long and there are three brakes on multiplication which eventually restrain it. They are the development of immunity in a population which restricts the duration of infectivity; the existence of previous infections in individuals receiving infective bites which may not therefore apparently produce new cases of the disease; and the occurrence of superinfection in the mosquito which may have previous infections so that subsequent ones do not materially increase its infectivity. These factors together reduce the gross reproduction rate to a net one which may be much lower. Should this fall below one, successive generations of cases would be smaller than their predecessors and the disease would disappear; should it be greater than one, successive generations would increase and the disease would mount in the population. Obviously the object of all control is to keep the reproduction rate below one so that successive generations decrease in size and the disease disappears.

Hence, there must be a *critical level of vector density* below which malaria tends to disappear and above which the incidence of the disease increases. This explains one type of the phenomenon of anophelism without

malaria. The other type is explained by the presence of nonvector anophelines.

All endemic malaria may be divided into two types—stable and unstable. *Stable malaria* occurs when the vector *Anopheles* species is strongly anthropophilic, has moderate to relatively great longevity, and lives in an environment with temperatures that favor rapid completion of the extrinsic cycle of the plasmodia. Relatively low critical vector densities are sufficient to maintain high endemicity. *Unstable malaria* occurs when the vector is relatively short-lived, less apt to feed on man, is in an environment with temperatures that at times are unfavorable to rapid parasite development. Relatively high critical densities are required to maintain transmission. But seasonal changes or unusual rainfall patterns frequently tend to increase vector densities to levels that result in epidemics, the human population being less protected by immunity than in areas of constant high endemicity. Classic examples of stable malaria are seen in *Anopheles gambiae* Giles areas of central Africa and unstable malaria is the rule in *Anopheles culicifacies* Giles areas of northwest India and of Ceylon. (9, 79, 111)

The new and useful quantitative look at the insect's role in malaria epidemiology makes it possible to improve certain entomological aspects of malaria surveys so as to obtain a more accurate picture of the integration of the several factors. Macdonald (79) sums up the present approach:

> The thread runs first through mosquito longevity, man-biting habit and period of extrinsic cycle to determine the stability of the disease; then through mosquito density and man-biting habit together with the normal reaction of the nonimmune to give a basic reproduction rate which determines initial transmission; then through climatic factors which determine the normal season; from these to an inoculation rate, and thence to communal immunity expressed in reduced gametocytaemia and reduced frequency of successful inoculations, and so to the net or locally ruling reproduction rate, in stable areas usually just over its critical level of 1.0 and in unstable ones vacillating partly erratically and partly periodically around it. This is the picture of malaria, and the process in reverse is the picture of its control.

Macdonald's text (79) not only gives full mathematical treatment of the subject in an appendix but also includes practical details for obtaining such now important entomological malaria-survey findings as basic and locally ruling reproduction rates, inoculation rates, vector man-biting habit, and longevity. Nonentomological aspects of malaria surveys are also included. This text is important to everyone interested in malaria and its control or in the newer aspects of epidemiology.

As previously noted, not much has been published yet about the newer survey methods. In addition to the studies by Davidson (20) and Davidson & Draper (21) cited above, there have been papers on the infectiousness of gametocytes by Draper (27) and by Jeffery & Eyles (55), exophily in *Anopheles gambiae* by Gillies (41), recognition of nulliparous female anoph-

elines by study of the mating plug by Gillies (42) and the statistical treatment of the sporozoite rate by Christie (12).

Anopheles Dispersal by Transportation

Hughes & Porter (50) have reviewed the subject of mosquito dispersal by means of various kinds of transportation. They note that *Anopheles subpictus indefinitus* Ludlow was found established in Guam in 1948. This is apparently the only instance of an anopheline mosquito's becoming established in the Central Pacific Micronesian area during and immediately after World War II. It is surmised that this insect may have entered Guam during military operations. To date it is not known to have transmitted any case of malaria. The authors cite several species of *Aëdes* and *Culex* that have been introduced into new areas, and they concluded that "we should remain constantly on the alert to prevent such importations, particularly in view of the emphasis that is being placed on mosquito control in this country and abroad. . . ."

The World Health Organization (132) reviewed the subject of the control of insect vectors in international air traffic, surveying the pertinent literature and existing legislation and practices. It was concluded that insect vectors of disease, including anophelines, can enter modern aircraft and survive long flights, and that "the spread of malaria in the world and the success or failure of its prevention and control can also be influenced by air transport."

Malaria Vectors

In Table 1, 63 species of *Anopheles* are listed which I consider to be vectors of malaria in one area or other of the world. Footnote † lists six species from the table that are suspect in areas not mentioned in the table; it includes also 16 species, not in the table, that at times or in certain places have been considered to be possible vectors.

In Table II are listed all the countries, territories, and mandated areas of the world, named by the 1956 United Nations Demographic Yearbook. For each country the status of malaria and malaria vectors is indicated.

Under references in each table it will be noted that the textbooks of Boyd (9), Faust & Russell (33), and Horsfall (49) have been cited frequently. These texts give many of the older references to the evidence on which the several species are classified as malaria vectors. Additional newer references are added in many cases. For a majority of the species listed there is clear-cut evidence from dissections that have revealed sporozoites. But sometimes, as in the case of Ethiopia, few or no dissections have been recorded and one must rely on the judgment of qualified epidemiologists who have made or have evaluated local surveys.

Status of Malaria

The data and reviews from which Tables I and II were prepared indicate that malaria has been retreating rapidly in North America since the

1880's, when the disease was endemic from southern Canada to the Isthmus of Panama. Now there is almost no malaria north of the United States-Mexican border, and malaria eradication is in progress in each of the Central American countries. Malaria may be said practically to have been eradicated from the continental United States, where in the 1930's as many as six or seven million cases occurred annually. In 1957 there were less than 10 proven cases of natural malaria transmission by mosquitoes. The two vectors, *Anopheles freeborni* Aitken in California and *Anopheles quadrimaculatus* Say in the Mississippi Valley and the southeastern United States are still prevalent, but human carriers are almost nonexistant. In Central America, malaria spread by *Anopheles albimanus* Weidemann, *aztecus* Hoffman, *darlingi* Root, *pseudopunctipennis* Theobald, or *punctimacula* Dyar & Knab is being eradicated by residual spraying with such insecticides as DDT, BHC, and dieldrin, and it seems reasonable to expect that the disease will be rooted out of North America within the next ten years or so.

In the West Indies in each country but Cuba there is a nationwide malaria eradication project in progress. The disease already has been eradicated from Puerto Rico and is at a low point in several wide areas once highly malarious. The West Indian vectors, *A. albimanus, Anopheles aquasalis* Curry, and *Anopheles argyritarsis* Robineau-Desvoidy present no special problem. But in Trinidad, *Anopheles bellator* Dyar & Knab, whose aquatic stages are found in water-holding tree-growing bromiliads, requires special control methods. Nonetheless, malaria is declining in Trinidad, and it appears that eradication is indeed possible.

So, too, in South America, despite a wide variety of vector anophelines, malaria eradication seems practicable, and in every malarious country a nationwide malaria eradication project is in progress. The disease already has been eradicated from Chile, from the coastal areas of British and French Guinea, and from most of Venezuela. Only a relatively small focus remains in Argentina. In Brazil, the bromiliad-breeding species, *A. bellator* and *Anopheles cruzii* Dyar & Knab, present special problems, not only because of the aquatic stage habitat, but because of outdoor resting and feeding habits.

As in North America, so in Europe; malaria has been retreating during the past 75 years. Hardly a country on the continent was free of this disease in the nineteenth century. Even Sweden, Finland, and northern Russia were subject to severe epidemics. But today no European country is seriously afflicted, the disease has been eradicated from Scandinavia, the United Kingdom, Baltic countries, and Germany, and practically from the Netherlands, Corsica, and Italy, including Sicily and Sardinia. No natural transmission has occurred on the latter island for several years. Only faint endemicity remains in Spain and Portugal, the Balkans, and the U.S.S.R. The vectors of the *maculipennis* complex—*Anopheles atroparvus* van Thiel, *Anopheles labranchiae* Falleroni, *Anopheles messae* Falleroni, and *Anopheles maculipennis* Meigen, as well as *Anopheles sacharovi* Favre and *Anopheles*

TABLE I

ANOPHELES MALARIA VECTOR SPECIES BY COUNTRIES OF THE WORLD*

Number	*Anopheles* Species	Countries	References
1	*aconitus* Dönitz	80, 81, 129, 190	9, 29, 33, 49, 114, 118, 119
2	*albimanus* Wiedemann	23, 26, 34, 41, 44, 45, 49, 50, 52, 71, 73, 74, 76, 107, 123, 131, 142, 189	9, 17, 33, 37, 38, 49, 98, 113, 123, 124
3	*albitarsis* Lynch-Arribalzaga	21, 26, 41, 133, 189	9, 17, 23, 33, 37, 38, 49, 105
4	*annularis* van der Wulp‖	29, 80	9, 29, 33, 49, 59, 101, 125
5	*aquasalis* Curry	21, 22, 26, 60, 71, 105, 166, 189	9, 23, 33, 35, 37, 38, 49
6	*argyritarsis* Robineau-Desvoidy	21, 71	113
7	*atroparvus* van Thiel‖	39, 66, 116, 138, 146, 161	9, 13, 33, 49
8	*aztecus* Hoffmann	107	26, 33, 49, 124
9	*baezai* Gater‡	102	48, 49
10	*bancrofti* Giles	193	29, 89, 90

* Numbers in the third column refer to countries listed and numbered in Table II; numbers in the fourth column refer to references listed and numbered in the bibliography.

† At times or in limited areas the following species of *Anopheles* have been suspected of being secondary malaria vectors (numbers refer to references): *A. adenensis* Christophers in Yemen (94); *A. algeriensis* Theobald in Algeria (49); *A. amictus* Edwards in Australia (49); *A. annulipes* Walker in Australasia (79); *A. brunnipes* (Theobald) in Belgian Congo (9); *A. crucians* Wiedemann in Cuba (49); *A. freeborni* Aitken in N.W. Mexico (9); *A. hispaniola* Theobald in Algeria and Morocco (49); *A. homunculus* Komp in Brazil; *A. jeyporiensis candidiensis* Koidzumi in India and on the Burmese border of Pakistan (129); *A. jeyporiensis jeyporiensis* James in India (129); *A. kochi* Dönitz in Indonesia (9); *A. maculatus* Theobald in the Philippines (49); *A. minimum flavirostris* (Ludlow) in W. Java (118); *A. multicolor* Cambouliu in Egypt (111); *A. pharöensis* Theobald in Aden (49); *A. plumbeus* Stephens in United Kingdom (116); *A. pretoriensis* (Theobald) in Saudi Arabia (personal communication); *A. pulcherrimus* Theobald in Iran and the U.S.S.R. (49); *A. ramsayi* Covell in India (49); *A. rhodesiensis* Theobald in Sierra Leone (9); *A. rivulorum* Leeson in Zanzibar (personal communication); *A. sergenti* (Theobald) in Yemen (94); *A. vestitipennis* Dyar & Knab in British Honduras, Cuba, and Mexico (9).

‡ The name "*umbrosus*" has been applied in the past to species now separately designated as *baezi, letifer,* and *umbrosus. Anopheles novumbrosus* is considered to be synonymous with *A. umbrosus.*

§ Sporozoites in *A. dureni* in the Belgian Congo may have been those of *Plasmodium berghei* Vincke & Lips, not a parasite of human malaria.

‖ *A. annularis* formerly *A. fuliginosus* Giles; *A. atroparvus* sometimes called *A. maculipennis atroparvus; A. claviger* formerly *A. bifurcatus* (Meigen); *A. fluviatilis* formerly *A. listoni* Liston; *A. labranchiae* sometimes called *A. maculipennis labranchiae; A. maculipennis maculipennis* sometimes called *A. maculipennis typicus; A. messae* sometimes called *A. maculipennis messae; A. nigerrimus* sometimes called *A. hyrcanus nigerrimus; A. sacharovi* sometimes called *A. maculipennis sacharovi,* formerly *A. elutus* Edwards; *A. sinensis* sometimes called *A. hyrcanus sinensis.*

TABLE I—(*Continued*)

Number	*Anopheles* Species	Countries	References
11	*barbirostris* van der Wulp	81, 102, 154	9, 29, 33, 48, 49, 134, 135
12	*bellator* Dyar & Knab	21, 26, 109	9, 33, 49, 105
13	*claviger* (Meigen)‖	46, 86, 95, 137, 183	9, 33, 49, 70, 112, 121
14	*cruzii* Dyar & Knab	21, 109	9, 33
15	*culicifacies* Giles	2, 29, 36, 39, 80, 82, 113, 115, 129, 140, 155	6, 9, 24, 29, 33, 49, 51, 62, 96, 109, 118
16	*darlingi* Root	9, 19, 21, 22, 23, 41, 60, 73, 113, 166, 189	9, 17, 23, 33, 35, 38, 49, 105
17	*d'thali* Patton	53	18
18	*dureni* Edwards§	16	49, 84
19	*farauti* Laveran	10, 24, 81, 119, 120, 132, 193	8, 33, 49, 87, 88, 89
20	*fluviatilis* James‖	80, 115, 129, 140, 155	9, 29, 33, 49, 60, 61, 62, 100, 109, 118
21	*freeborni* Aitken†	185	9, 11, 33, 49
22	*funestus* Giles	8, 14, 16, 31, 32, 53, 59, 62, 63, 64, 65, 91, 96, 101, 112, 124, 145, 147, 157, 159, 165, 169, 173, 175, 176, 181, 182, 197	9, 19, 21, 28, 33, 40, 47, 49, 67, 84, 99
23	*gambiae* Giles	1, 8, 16, 25, 31, 32, 35, 42, 53, 59, 62, 63, 64, 65, 91, 96, 101, 106, 112, 124, 139, 144, 145, 147, 153, 155, 157, 159, 160, 162, 165, 169, 173, 178, 181, 182, 195, 197	5, 9, 19, 21, 28, 33, 39, 40, 47, 49, 66, 67, 80, 99
24	*gambiae melas* Theobald	64, 96, 124, 157	5, 33, 39, 49, 84, 95, 99
25	*hancocki* Edwards	32, 96, 124, 157, 181	9, 33, 46, 49, 68, 84, 99
26	*hargreavesi* Evans	124	9, 33, 49, 84
27	*jeyporiensis candidiensis*† Koidzumi	29, 30, 39, 77, 94, 190	9, 33, 39, 49, 118, 129
28	*karwari* (James)	193	9, 49, 86, 88, 89, 90
29	*koliensis* Owen	193	87, 88, 89, 90
30	*labranchiae* Falleroni‖	5, 58, 87, 111, 161, 179	9, 33, 49, 109
31	*letifer* Gater‡	81, 102	29, 48
32	*leucosphyrus* Dönitz	29, 30, 80, 81, 154	9, 16, 33, 49, 63, 102, 134, 135
33	*leucosphyrus balabacensis* Baisas	27, 127	33, 81, 134
34	*maculatus* Theobald†	29, 30, 39, 81, 94, 102, 158, 174, 190	9, 22, 29, 33, 48, 49, 118
35	*maculipennis maculipennis* Meigen‖	69, 82, 83, 183	33, 49, 103, 104
36	*mangyanus* (Banks)	135	29, 33, 109

TABLE I—(*Continued*)

Number	*Anopheles* Species	Countries	References
37	*messae* Falleroni‖	4, 11, 28, 47, 78, 137, 146, 183, 196	9, 33, 49, 121
38	*minimus* Theobald	18, 29, 30, 39, 77, 80, 81, 92, 94, 100, 115, 129, 174, 190	4, 9, 33, 49, 64, 91, 92, 102, 118, 119, 122, 133
39	*minimus flavirostris* (Ludlow)†	135	9, 29, 30, 33, 49, 109
40	*moucheti* Evans	16, 32, 181	1, 2, 9, 33, 44, 49
41	*multicolor* Cambouliu†	5, 111, 179	49, 97
42	*nigerrimus* Giles‖	81, 90	9, 29, 33, 49
43	*nili* (Theobald)	16, 32, 63, 96, 157, 165	2, 9, 47, 66, 67, 84, 95, 99
44	*nuñez-tovari* Gabaldon	41, 189	17, 37
45	*pattoni* Christophers	39	49, 79
46	*pharöensis* Theobald†	51, 53, 63, 124, 181	9, 18, 33, 47, 49, 84
47	*philippinensis* Ludlow	29, 80, 129	9, 33, 49, 58, 102, 118
48	*pseudopunctipennis* Theobald	9, 19, 38, 41, 50, 52, 73, 107, 123, 134	9, 17, 33, 49, 124
49	*punctimacula* Dyar & Knab	34, 41, 50, 131, 134	9, 17, 33, 49
50	*punctulatus* Dönitz	24, 119, 132, 193	9, 33, 44, 49, 87 to 90
51	*quadrimaculatus* Say	185	9, 11, 33, 49
52	*rufipes* (Gough)	63, 165	33, 47, 49
53	*sacharovi* Favre‖	4, 28, 39, 46, 58, 69, 82, 83, 86, 87, 90, 95, 146, 172, 180	9, 13, 14, 33, 49, 103, 104, 112
54	*sergenti* (Theobald)†	5, 51, 86, 90, 97, 111, 155, 172, 179	9, 31, 33, 49, 112
55	*sinensis* Wiedemann‖	30, 39, 88, 92, 94, 100, 148, 190	9, 33, 49, 56, 133
56	*stephensi* Liston	12, 80, 82, 83, 113, 129, 155	9, 33, 49, 59, 60, 103, 104, 118
57	*stephensi mysorensis* Sweet & Rao	80	59, 60, 118
58	*subpictus* Grassi	81, 190	9, 49
59	*sundaicus* (Rodenwaldt)	29, 30, 80, 81, 102, 129, 158, 174, 190	9, 33, 48, 49, 101, 118, 127
60	*superpictus* Grassi	2, 4, 28, 46, 69, 82, 83, 86, 87, 90, 95, 129, 172, 180, 183, 196	9, 24, 33, 34, 49, 52, 103, 104, 112, 121
61	*tesselatus* Theobald	103	9, 33, 49, 54
62	*umbrosus* (Theobald)‡	102	9, 33, 48, 49
63	*varuna* Iyengar	80	9, 33, 49, 118, 126

TABLE II

MALARIA AND MALARIA VECTORS IN THE COUNTRIES AND TERRITORIES OF THE WORLD*

Number	Country or Territory	Status of Malaria	Malaria Vectors	References
1	Aden and Protectorate†	P	23	9
2	Afghanistan	P	15, 60	24, 29, 34, 52
3	Alaska	N		
4	Albania	MEC	37, 53, 60	9, 49
5	Algeria†	P	30, 41, 54	9, 97
6	American Samoa	N		
7	Andorra	N		
8	Angola	HP	22, 23	9, 49
9	Argentina	MEC	16, 48	9, 33, 49
10	Australia†	FP	19	48, 83, 90
11	Austria	N		
12	Bahrein	P	56	9, 118
13	Basutoland	N		
14	Bechuanaland	P	22	84
15	Belgium	N		
16	Belgian Congo†	HP	18 (?), 22, 23, 40, 43	9, 49, 84, 95
17	Bermuda	N		
18	Bhutan	P	38	64
19	Bolivia	MEC	16, 48	9
20	Bonin Islands	N		
21	Brazil†	MEC	3, 5, 6, 12, 14, 16	9, 23, 33, 49, 105
22	British Guiana	MEC	5, 16	9, 49
23	British Honduras†	MEC	2, 16	9, 49
24	British Solomon Islands	HP	19, 50	49, 53, 90
25	British Somaliland	HP	23	9
26	British West Indies	MEC	2, 3, 5, 12	33, 49
	Bahama Islands	N		
	Barbados	N		
	Jamaica	MEC	2	33, 49
	Leeward Islands	MEC	2, 5	33
	Trinidad & Tobago	MEC	3, 5, 12	49
	Windward Islands	MEC	5	33

* List of countries from 1956 United Nations Demographic Yearbook; numbers in the fourth column refer to *Anopheles* vector species listed and numbered in Table I; numbers in the fifth column refer to references listed and numbered in the bibliography; symbols in the third column signify the present status of malaria, as follows: N=not endemic; FP=faintly endemic; P=moderately endemic; HP=highly endemic; ME=eradicated since World War II; MEC=nationwide malaria eradication project in progress.

† See footnote † of Table I. ‡ Corsica.

§ *Anopheles gambiae* in Canary Islands of Spain

‖ *Anopheles funestus* appears to have been eradicated from Mauritius.

TABLE II—(*Continued*)

Number	Country or Territory	Status of Malaria	Malaria Vectors	References
27	Brunei	MEC	33	134
28	Bulgaria	MEC	37, 53, 60	9
29	Burma	MEC	4, 15, 27, 32, 38, 47, 59	9, 29, 49, 101, 102, 118
30	Cambodia	MEC	27, 32, 38, 55, 59	9, 49, 118
31	Cameroons, British	HP	22, 23	
32	Cameroons, French	HP	22, 23, 25, 40, 43	1, 2, 47, 66, 84
33	Canada	N		
34	Canal Zone	MEC	2, 49	9
35	Cape Verde Islands	P	23	85
36	Ceylon	MEC	15	9, 29, 49
37	Channel Islands	N		
38	Chile	ME	48	9, 49
39	China Mainland	HP	4, 7, 15, 27, 38, 45, 53, 55	49, 118, 120
	Taiwan (Formosa)	MEC	38, 55	29, 49, 122, 133
40	Cocos (Keeling) Islands	N		
41	Columbia	MEC	2, 3, 16, 44, 48, 49	9, 17, 33, 49
42	Comoro Islands	HP	23	9
43	Cook Island	N		
44	Costa Rica	MEC	2	49
45	Cuba†	P	2	49
46	Cyprus	ME	15, 53, 60	9, 49
47	Czechoslovakia	MEC	37	9, 33
48	Denmark	N		
49	Dominican Republic	MEC	2	123
50	Ecuador	MEC	2, 48, 49	9, 33
51	Egypt†	MEC	46, 54	9, 31, 84
52	El Salvador	MEC	2, 48	49
53	Ethiopia & Eritrea, Federation	HP	17, 22, 23, 46	9, 18, 49
54	Falkland Islands	N		
55	Faroe Islands	N		
56	Fiji Islands	N		
57	Finland	N		
58	France‡	MEC	30	33
59	French Equatorial Africa	HP	22, 23	9, 47
60	French Guiana	MEC	5, 16	9, 35, 49
61	French Oceania	N		
62	French Somaliland	HP	22, 23	
63	French West Africa	HP	22, 23, 43, 46, 52	9, 47, 49
64	Gambia	HP	22, 23, 24	5, 49, 84
65	Ghana	HP	22, 23	15, 49, 84
66	Germany	ME	7	9
67	Gibraltar	N		

TABLE II—(*Continued*)

Number	Country or Territory	Status of Malaria	Malaria Vectors	References
68	Gilbert & Ellice Islands	N		
69	Greece	MEC	35, 53, 60	9, 33
70	Greenland	N		
71	Guadaloupe	MEC	2, 5, 6	113
72	Guam	N		
73	Guatemala	MEC	2, 16, 48	9
74	Haiti	MEC	2	49
75	Hawaii	N		
76	Honduras	MEC	2	49
77	Hong Kong	FP	27, 28	49, 118
78	Hungary	MEC	37	9
79	Iceland	N		
80	India†	MEC	1, 4, 15, 20, 32, 38, 47, 57, 59, 63	6, 29, 49, 58 to 64, 91, 92, 114, 115, 118, 125, 126, 127, 129
81	Indonesia†	MEC	1, 4, 11, 19, 31, 32, 34, 38, 42, 58, 59	29, 49, 118
82	Iran†	MEC	15, 35, 53, 56, 60	9, 33
83	Iraq	MEC	35, 53, 56, 60	9, 33, 103, 104
84	Ireland	N		
85	Isle of Man	N		
86	Israel	MEC	13, 53, 54, 60	31, 112
87	Italy	ME	30, 53, 60	9, 33, 49
88	Japan	FP	55	9, 33, 56
89	Johnston Island	N		
90	Jordan	MEC	53, 54, 60	9, 31
91	Kenya	HP	22, 23	9, 49, 84
92	Korea	P	38, 55	33
93	Kuwait	N		
94	Laos	MEC	27, 38, 55	9, 49, 118
95	Lebanon	MEC	13, 53, 60	9, 31, 33
96	Liberia	HP	22, 23, 24, 25, 43	36, 39, 49, 84, 89, 99
97	Libya	P	54	
98	Liechtenstein	N		
99	Luxembourg	N		
100	Macao	P	38, 55	
101	Madagascar	MEC	22, 23	9, 43, 49
102	Malaya, Federation of	HP	9, 11, 31, 34, 59, 62	22, 29, 48, 49, 118
103	Maldive Islands	P	61	54
104	Malta & Gozo	N		
105	Martinique	MEC	5	9

TABLE II—(*Continued*)

Number	Country or Territory	Status of Malaria	Malaria Vectors	References
106	Mauritius‖	ME	22, 23	9, 25, 45, 49
107	Mexico†	MEC	2, 8, 48	9, 26, 33, 49, 98. 124
108	Midway Island	N		
109	Monaco	N		
110	Mongolian People's Republic	N		
111	Morocco†	P	30, 41, 54	9
112	Mozambique	HP	22, 23	9
113	Muscat & Oman	HP	15, 56	9
114	Nauru Island	N		
115	Nepal	P	15, 20, 38	100
116	Netherlands	MEC	7	9, 49
117	Netherlands Antilles	N		
118	New Caledonia	N		
119	New Guinea	HP	19, 50	49, 53, 90
120	New Hebrides	HP	19	53, 65, 90
121	New Zealand	N		
122	New Zealand Oceania	N		
123	Nicaragua	MEC	2, 48	9
124	Nigeria, Federation of	HP	22, 23, 24, 25, 26 46	3, 10, 47, 49, 84, 95
125	Niue Island	N		
126	Norfolk Island	N		
127	North Borneo	MEC	33	49, 81, 82, 134
128	Norway	N		
129	Pakistan	HP	15, 20, 38, 47, 56, 59, 60	49, 51, 96, 118
130	Palestine—Gaza Strip	ME	13	9
131	Panama	MEC	2, 49	49
132	Papua	HP	19, 50	7, 8, 44
133	Paraguay	MEC	3, 16	9
134	Peru	MEC	48, 49	9, 49
135	Philippines†	MEC	36, 39	29, 30, 49
136	Pitcairn Island	N		
137	Poland	FP	13, 37	9, 70
138	Portugal	FP	7	9, 33
139	Portuguese Guinea	HP	23	47
140	Portuguese India	FP	15, 20	29
141	Portuguese Timor	P	11, 59	
142	Puerto Rico	ME	2	9
143	Qatar	N		
144	Reunion	FP	23	9, 46
145	Rhodesia & Nyassaland, Federation	HP	22, 23	9, 49, 84, 106

TABLE II—(*Continued*)

Number	Country or Territory	Status of Malaria	Malaria Vectors	References
146	Romania	MEC	7, 37, 53	9, 13, 14
147	Ruanda-Urundi	HP	22, 23	84
148	Ryukyu Islands	FP	55	
149	Saar	N		
150	St. Helena	N		
151	St. Pierre & Miquelon	N		
152	San Marino	N		
153	São Tomé & Principe	P	23	9
154	Sarawak	MEC	11, 32	16, 29, 49, 134, 135
155	Saudi Arabia†	HP	15, 20, 23, 54, 56	9, 32
156	Seychelles	N		
157	Sierra Leone†	HP	22, 23, 24, 25, 43	49, 68, 84
158	Singapore	FP	34, 59	48
159	Somaliland, Italian	HP	22, 23	9, 93
160	South West Africa	P	23	
161	Spain§	FP	7, 23, 30	9, 33
162	Spanish Guinea	HP	23	
163	Spanish North Africa	FP	?	
164	Spanish West Africa	FP	?	
165	Sudan	HP	22, 23, 43, 52	9, 49, 67, 84
166	Surinam	MEC	5, 16	9
167	Svalbard & Jan Mayen Islands	N		
168	Swan Islands	N		
169	Swaziland	MEC	22, 23	80
170	Sweden	N		
171	Switzerland	N		
172	Syria	MEC	53, 54, 60	9, 31, 33
173	Tanganyika	HP	22, 23	9, 21, 28, 40, 49
174	Thailand	MEC	34, 38, 59	4, 9, 29, 49, 118
175	Togoland, British	HP	22	
176	Togoland, French	HP	22	
177	Tokelau Island	N		
178	Trucial Oman	HP	23	94
179	Tunisia	P	30, 41, 54	9
180	Turkey	MEC	53, 60	9, 57
181	Uganda	HP	22, 23, 25, 40, 46	9, 19, 49, 84
182	Union of South Africa	MEC	22, 23	49, 84
183	Union of Soviet Socialist Republic†	MEC	13, 35, 37, 60	9, 49, 121
184	United Kingdom, Great Britain Northern Ireland†	N		
185	United States of America	ME	21, 51	9, 11, 49
186	United States Pacific Islands	N		
187	Uruguay	N		

TABLE II—(*Continued*)

Number	Country or Territory	Status of Malaria	Malaria Vectors	References
188	Vatican	N		
189	Venezuela	MEC	2, 3, 5, 16, 44	9, 37, 38, 49
190	Vietnam	MEC	1, 27, 38, 55, 58, 59	9, 29, 49, 118, 119
191	Virgin Islands	N		
192	Wake Island	N		
193	West New Guinea	HP	10, 19, 28, 29, 50	7, 29, 86 to 90
194	Western Samoa	N		
195	Zemen†	HP	23	94
196	Yugoslavia	MEC	37, 60	9, 117
197	Yanzibar & Pemba†	MEC	22, 23	49

superpictus Grassi, are all readily controlled by residual spraying, except in Greece, where *A. sacharovi* is now resistant. Here recourse to malaria drugs will doubtless solve the problem and eliminate the relatively small focus of malaria that remains in that once highly malarious country. It seems reasonable to suppose that malaria will disappear from Europe within the next ten years or so.

In the Near East, malaria has been eradicated from Cyprus, and is at a low point in Iran, Lebanon and Turkey. There are malaria eradication projects in progress in Iran, Iraq, Israel, Jordan, Lebanon, Syria, and Turkey. The chief vectors, *Anopheles claviger* (Meigen), *Anopheles maculipennis maculipennis* Meigen, *A. sacharovi*, *Anopheles sergenti* (Theobald), *Anopheles stephensi* Liston, and *A. superpictus*, are—all but *A. sergenti*—fairly easily controlled by residual spraying. *A. sergenti* in Jordan and Israel rests and feeds mostly out-of-doors and therefore is not much affected by residual spraying. It has been successfully controlled in some areas of Jordan by residual dieldrin larviciding. *A. stephensi* in southern Iran and Iraq is now resistant to DDT but is killed by dieldrin.

Malaria remains a problem in the Arabian area. Good control has been accomplished in only a few places, as in Dhahran. Here *A. stephensi* is now resistant to DDT but is killed by dieldrin. *A. gambiae* is the chief vector in west Arabia, *A. culicifacies*, *fluviatilis*, *sergenti*, and *stephensi* in the east.

Africa presents a picture full of contrasts as regards insects and the epidemiology of malaria. In the central part of the range of the two chief vectors (*Anopheles funestus* Giles and *A. gambiae*), between the Tropics of Cancer and Capricorn, very little progress has been made toward control, and there are no large areas where residual spraying has been demonstrated successfully and none where malaria has been eradicated. Yet on the periphery of this range, malaria is being dealt with effectively. In Mauritius, it has been eradicated and *A. funestus* has disappeared. *A.*

gambiae remains in large numbers but is much less often taken inside houses. In areas of Madagascar, South Africa, Southern Rhodesia, and Swaziland, malaria is retreating rapidly in response to residual spraying. Malaria eradication projects are in progress in Madagascar, Swaziland, and Zanzibar. Good results have also been reported from residual spraying in the highlands of Ethiopia. In North Africa, malaria remains endemic in Morocco, Algeria, Tunisia, and Libya but would seem to present no serious technical problems of eradication. Egypt is carrying out a nationwide malaria eradication project with every prospect of success. *A. gambiae* has become resistant to dieldrin in Nigeria but is killed by DDT.

In middle and far-eastern Asia there are now in progress nationwide malaria eradication projects in Brunei, Burma, Cambodia, Ceylon, India, Indonesia, Laos, North Borneo, Philippines, Sarawak, Thailand, Taiwan, and Vietnam. The disease has already been eradicated from wide areas of Ceylon, Taiwan, and Thailand. There seem to be no serious technical problems in regard to the control of any of the many Asiatic anopheline vectors. *Anopheles sundaicus* (Rodenwaldt) is resistant to DDT in some areas of northern Java but is killed by dieldrin. The largest malaria eradication project in the world was inaugurated in India in April, 1958. Here over 300 millions live in malarious areas and will come under the project. The excellent results of a five-year India National Malaria Control Programme, just ended, give good promise of success.

Malaria is only faintly endemic in Australia but is still highly prevalent in New Guinea, New Hebrides, Papua, British Solomon Islands, and West New Guinea. The chief vectors are *Anopheles farauti* Laveran and *Anopheles punctulatus* Dönitz.

LITERATURE CITED

1. Adam, J. P., *Parasitáire humáine et comparée*, **30,** 389–94 (1955)
2. Adam, J. P., *Bull. soc. pathol. exotique*, **49,** 210–20 (1956)
3. Archibald, H. M., *Bull. World Health Organization*, **15,** 695–709 (1956)
4. Ayurakitkosol, L., and Griffith, M. E., *Bull. World Health Organization*, **15,** 799–805 (1956)
5. Bertram, D. S., and McGregor, I. A., *Bull. Entomol. Research*, **47,** 669–81 (1956)
6. Bhatia, M. L., and Krishnan, K. S., *Bull. Natl. Soc. India Malaria (Delhi)*, **5,** 1–34 (1957)
7. Black, R. H., *South Pacific Comm. Tech. Paper No. 61* (1954)
8. Black, R. H., *Med. J. Australia*, **42,** 949–55 (1955)
9. Boyd, M. F., *Malariology* (W. B. Saunders Co., Philadelphia, Pa., 1643 pp., 1949)
10. Bruce-Chwatt, L. J., *Trans. Roy. Soc. Trop. Med. Hyg.*, **51,** 411–18 (1957)
11. Carpenter, S. J., and La Casse, W. J., *Mosquitoes of North American (North of Mexico)* (University of California Press, Berkeley, Calif., 360 pp., 1955)
12. Christie, M., *Ann. Trop. Med. Parasitol.*, **50,** 350–54 (1956)
13. Ciuca, M., *Bull. World Health Organization*, **15,** 725–51, (1956)
14. Ciuca, M., *Trop. Diseases Bull.*, **56,** 1389–90 (1957)
15. Colburne, M. J., and Wright, F. N., *W. African Med. J.*, **4,** 3–17. 161–74 (1955)

16. Colless, D. H., *Bull. Entomol. Research*, **47**, 115–23 (1956)
17. Concha y Venegas, J. A., *Z. Tropenmed. Parasitol.*, **6**, 336–43 (1955)
18. Covell, G., *J. Trop. Med. Hyg.*, **60**, 7–16 (1957)
19. Davidson, G., *Trans. Roy. Soc. Trop. Med. Hyg.*, **49**, 339–50 (1955)
20. Davidson, G., *Ann. Trop. Med. Parasitol.*, **48**, 58–74 (1954)
21. Davidson, G., and Draper, C. C., *Trans. Roy. Soc. Trop. Med. Hyg.*, **47**, 522–35 (1953)
22. Davidson, G., and Ganapathipillai, A., *Ann. Trop. Med. Parasitol.*, **50**, 137–46 (1956)
23. Deane, L. M., Causey, O. R., and Deane, M. P., *Studies on Brazilian Anophelines from the Northeast and Amazon Regions* (Johns Hopkins Press, Baltimore, Md., 50 pp., 1946)
24. Dhir, S. L., and Rahim, A., *Indian J. Malariol.*, **11**, 73–126 (1957)
25. Dowling, M. A. C., *Trans. Roy. Soc. Trop. Med. Hyg.*, **47**, 177–98 (1953)
26. Downs, W. G., and Bordas, E., *J. Natl. Malaria Soc.*, **10**, 350–58 (1951)
27. Draper, C. C., *Trans. Roy. Soc. Trop. Med. Hyg.*, **47**, 160–65 (1953)
28. Draper, C. C., and Smith, A., *Trans. Roy. Soc. Trop. Med. Hyg.*, **51**, 137–51 (1957)
29. Dy, F. J., *Bull. World Health Organization*, **11**, 725–63 (1954)
30. Ejercito, A., Hess, A. D., and Willard, A., *Am. J. Trop. Med. Hyg.*, **3**, 971–80 (1954)
31. Farid, M. A., *Bull. World Health Organization*, **15**, 821–28 (1956)
32. Farid, M. A., *Bull. World Health Organization*, **15**, 828–33 (1956)
33. Faust, E. C., and Russell, P. F., *Clinical Parasitology* (Lee & Febiger, Philadelphia, Pa., 1078 pp., 1957)
34. Fischer, L., and Steinhart, W., *Z. Tropenmed. Parasitol.*, **8**, 69–83 (1957)
35. Floch, H., *Bull. World Health Organization*, **11**, 579–633 (1954)
36. Fox, R. M., *Am. J. Trop. Med. Hyg.*, **6**, 598–620 (1957)
37. Gabaldon, A., *Am. J. Trop. Med. Hyg.*, **5**, 966–76 (1956)
38. Gabaldon, A., and Berti, A. L., *Am. J. Trop. Med. Hyg.*, **3**, 793–807 (1954)
39. Gelfand, H. M., *Trans. Roy. Soc. Trop. Med. Hyg.*, **49**, 508–27 (1955)
40. Gillies, M. T., *Ann. Trop. Med. Parasitol.*, **48**, 58–74 (1954)
41. Gillies, M. T., *Bull. World Health Organization*, **15**, 437–49 (1956)
42. Gillies, M. T., *Bull. World Health Organization*, **15**, 451–59 (1956)
43. Grjebine, A., *Bull. World Health Organization*, **15**, 593–611 (1956)
44. Gunther, C. E. M., *Med. J. Australia*, **1**, 493–98 (1956)
45. Halcrow, J. G., *Nature*, **177**, 1103–5 (1956)
46. Hamon, J., and Dufour, G., *Bull. World Health Organization*, **11**, 525–56 (1954)
47. Hamon, J., Adam, J. P., and Grjebine, A., *Bull. World Health Organization*, **15**, 549–91 (1956)
48. Hodgkin, E. P., *The Transmission of Malaria in Malaya* (Institute of Medical Research, Federation of Malaya, Kuala Lumpur, 98 pp., 1956)
49. Horsfall, W. R., *Mosquitoes, Their Bionomics and Relation to Disease* (Ronald Press Co., New York, N.Y., 723 pp., 1955)
50. Hughes, J. H., and Porter, J. E., *Mosquito News*, **16**, 106–11 (1956)
51. Husain, M. Z. Y., and Talibili, S. A., *Pakistan J. Health*, **6**, 65–72 (1956)
52. Iyengar, M. O. T., *Trans. Roy. Soc. Trop. Med. Hyg.*, **48**, 319–24 (1954)
53. Iyengar, M. O. T., *South Pacific Comm. Tech. Paper No. 86* (1955)
54. Iyengar, M. O. T., Mathew, M. I., and Menon, M. A. U., *Indian J. Malariol.*, **7**, 1–3 (1953)

55. Jeffery, G. M., and Eyles, D. E., *Am. J. Trop. Med. Hyg.*, **4**, 781–89 (1955)
56. Kobayashi, H., *Nagasaki Med. Soc. J.*, **30**, 365–94 (1955)
57. Kratz, F. W., and Bridges, C. B., *Public Health Repts. (U.S.)*, **71**, 409–16 (1956)
58. Krishnan, K. S., *Bull. Natl. Soc. India Malaria (Delhi)*, **1**, 148–55 (1953)
59. Krishnan, K. S., *Bull. Natl. Soc. India Malaria (Delhi)*, **2**, 98–106 (1954)
60. Krishnan, K. S., *Bull. Natl. Soc. India Malaria (Delhi)*, **2**, 183 (1954)
61. Krishnan, K. S., *Bull. Natl. Soc. India Malaria (Delhi)*, **2**, 184–85 (1954)
62. Krishnan, K. S., *Bull. Natl. Soc. India Malaria (Delhi)*, **3**, 93–94 (1955)
63. Krishnamurthy, B. S., *Bull. Natl. Soc. India Malaria (Delhi)*, **3**, 1–8 (1955)
64. Krishnaswami, A. K., *Bull. Natl. Soc. India Malaria (Delhi)*, **1**, 157–63 (1953)
65. Laird, M., *Bull. Entomol. Research*, **45**, 279–83 (1954)
66. Languillon, J., Mouchet, J., Rivola, E., and Rateau, J., *Méd. trop.*, **16**, 347–78 (1956)
67. Lewis, D. J., *Bull. Entomol. Research*, **47**, 475–94 (1956)
68. Lewis, D. J., *Ann. Trop. Med. Parasitol.*, **50**, 299–313 (1956)
69. Lotka, A. J., *Am. J. Hyg.*, **3**, 113–21 (1923)
70. Lukasiak, J., *Trop. Diseases Bull.*, **54**, 1286–87 (1957)
71. Macdonald, G., *Trop. Diseases Bull.*, **47**, 907–15 (1950)
72. Macdonald, G., *Trop. Diseases Bull.*, **47**, 915–38 (1950)
73. Macdonald, G., *Trop. Diseases Bull.*, **49**, 569–86 (1952)
74. Macdonald, G., *Trop. Diseases Bull.*, **49**, 813–29 (1952)
75. Macdonald, G., *Trop. Diseases Bull.*, **50**, 871–89 (1953)
76. Macdonald, G., *Proc. Roy. Soc. Med.*, **48**, 295–301 (1955)
77. Macdonald, G., *Bull. World Health Organization*, **15**, 369–87 (1956)
78. Macdonald, G., *Bull. World Health Organization*, **15**, 613–26 (1956)
79. Macdonald, G., *The Epidemiology and Control of Malaria* (Oxford University Press, London, England, 201 pp., 1957)
80. Mastbaum, O. J., *Trop. Med. Hyg.*, **60**, 119–27, 190–92 (1957)
81. McArthur, J., *Trans. Roy. Soc. Trop. Med. Hyg.*, **40**, 537–58 (1947)
82. McArthur, J., *Indian J. Malariol.*, **4**, 1–90 (1950)
83. McKerras, M. J., and Sanders, D. F., *South Pacific Comm. Tech. Paper No. 68* (1954)
84. Meillon, B. de, *The Anophelini of the Ethiopian Geographical Region* (South African Institute of Medical Research, Johannesburg, 272 pp., 1947)
85. Meira, M. T. V. de, *Anais inst. Med. trop.*, **11**, 287–338 (1954)
86. Metselaar, D., *Documenta Med. Geograph. et Trop.*, **7**, 193–96 (1955)
87. Metselaar, D., *Documenta Med. Geograph. et Trop.*, **8**, 363–64 (1956)
88. Metselaar, D., *Bull. World Health Organization*, **15**, 635–49 (1956)
89. Metselaar, D., *Acta Leidensia*, **27**, 1–128 (1957)
90. Metselaar, D., *Pilot Project of Residual Insecticide Spraying in Netherlands New Guinea* (Kemink en Zoon N. V., Utrecht, Netherlands, 128 pp., 1957)
91. Misra, B. G., *Bull. Natl. Soc. India Malaria (Delhi)*, **2**, 151–52 (1954)
92. Misra, B. G., *Indian J. Malariol.*, **10**, 331–47 (1956)
93. Moise, R., *Riv. malariol.*, **32**, 11–39 (1953)
94. Mount, R. A., *Am. J. Trop. Med. Hyg.*, **2**, 1–12 (1953)
95. Muirhead-Thomson, R. C., *Mosquito Behavior in Relation to Malaria Transmission and Control in the Tropics* (Ed. Arnold & Co., London, England, 219 pp., 1951)
96. Naqvi, S. H., and Qutub-ud-Din, M., *Pakistan J. Health*, **3**, 241–53 (1954)
97. Passager, P., *Arch. inst. Pasteur Algérie*, **35**, 99–200 (1957)

98. Pesqueira, M. E., *Bol. epidemiol. (Mexico)*, **20,** 10–17 (1956)
99. Peters, W., *Bull. Entomol. Research*, **47,** 525–51 (1956)
100. Peters, W., Dewar, S. C., Bhalla, B. D., and Manadhar, T. L., *Indian J. Malariol.*, **9,** 207–12 (1955)
101. Postiglione, M., *Bull. Natl. Soc. India Malaria (Delhi)*, **4,** 65–70 (1956)
102. Postiglione, M., and Venkat Rao, V., *Indian J. Malariol.*, **10,** 273–98 (1956)
103. Pringle, G., *Bull. Endemic Diseases (Baghdad)*, **1,** 2–45 (1954)
104. Pringle, G., *Bull. Endemic Diseases (Baghdad)*, **1,** 87–117; 187–236 (1955)
105. Rachou, R. G., *Rev. brasil. malariol. e doenças trop.*, **6,** 177–88 (1954)
106. Reid, E. T., and Woods, R. W., *Proc. and Trans. Rhodesia Sci. Assoc.*, **45,** 47–72 (1957)
107. Ross, R., *The Prevention of Malaria* (John Murray, London, England, 711 pp., 1911)
108. Ross, R., *Proc. Roy. Soc. (London)*, [A]**92,** 204–30 (1916)
109. Russell, P. F., *Malaria; Basic Principles Briefly Stated* (Blackwell Scientific Publications, Oxford, England, 210 pp., 1952)
110. Russell, P. F., *Man's Mastery of Malaria* (Oxford University Press, London, England, 308 pp., 1955)
111. Russell, P. F., West, L. S., and Manwell, R. D., *Practical Malariology* (W. B. Saunders Co., Philadelphia, Pa., 684 pp., 1946)
112. Salternik, Z., *Trop. Diseases Bull.*, **54,** 9–10 (1957)
113. Sautet, J., and Aldighieri, R., *Bull. soc. pathol. exotique*, **47,** 387–91 (1954)
114. Senior-White, R., Adhikari, A. K., Ramakrishna, V., and Roy, B. B., *J. Malaria Inst. India*, **5,** 159–86 (1943)
115. Sharma, M. I. D., *Bull. Natl. Soc. India Malaria (Delhi)*, **2,** 31–45 (1954)
116. Shute, P. G., *Monthly Bull. Ministry Health Lab. Serv. (United Kingdom)* **13,** 48–51 (1954)
117. Simič, Č., *Bull. World Health Organization*, **15,** 753–66 (1956)
118. Singh, J., Pal, R., and Bhatia, M. L., *Bull. Natl. Soc. India Malaria (Delhi)*, **2,** 31–45 (1954)
119. Soulage, J., and Richet, P., *Méd. trop.*, **13,** 617–40 (1953)
120. Sweet, W. C., Feng, L. C., Chow, C. Y., and Hsu, S. C., *J. Natl. Malaria Soc.*, **1,** 25–32 (1942)
121. Trofimov, G. K., and Lysenko, V. F., *Med. Parazitol. Parazitar. Bolezni*, **24,** 26–30 (1955)
122. Tseng, P., and Hsieh, H., *Tai-wan i-hsüeh-hui tsa-chih*, **53,** 568–72 (1954)
123. Ureña Hernandez, L., and Gañan, D., *Rev. med. dominicana*, **8,** 66–84 (1953)
124. Vargas, L., and Palacios, M., *Rev. inst. salubridad y enfermendad. trop. (Mex.)*, **15,** 81–123 (1955)
125. Venkat Rao, V., *Bull. Natl. Soc. India Malaria (Delhi)*, **2,** 191–99 (1954)
126. Venkat Rao, V., *Bull. Natl. Soc. India Malaria (Delhi)*, **3,** 9–23 (1955)
127. Venkat Rao, V., *Bull. Natl. Soc. India Malaria (Delhi)*, **4,** 27–35 (1956)
128. Waite, H., *Biometrika*, **7,** 421–36 (1910)
129. Wattal, B. L., *Bull. Natl. Soc. India Malaria (Delhi)*, **5,** 177–90 (1957)
130. Weitz, B., *J. Hyg.*, **50,** 275–94 (1952)
131. Weitz, B., *Bull. World Health Organization*, **15,** 473–90 (1956)
132. World Health Organization, *Control of Insect Vectors in International Air Traffic* (World Health Organization, Geneva, Switzerland, 59 pp., 1955)
133. Wu, Y. T., *Tai-wan i-hsüch-hui tsa-chih*, **55,** 491–501 (1956)
134. Zulueta, J. de, *Bull. World Health Organization*, **15,** 651–71 (1956)
135. Zulueta, J. de, *Bull. World Health Organization*, **15,** 673–93 (1956)

AUTHOR INDEX

A

Ackerman, L., 62
Acree, F., Jr., 43
Adam, J. P., 423, 424, 425, 426, 428
Adams, C. C., 102
Adamson, A. M., 183
Adamson, B. E., 193
Adhikari, A. K., 422, 427
Adriaanse, A., 94, 227
Ahmad, T., 238
Aizawa, K., 34
Åkesson, B., 5, 6, 10
Albert, A., 65, 66
Albrecht, A. M., 53
Aldighieri, R., 422, 426
Aldridge, W. N., 310, 313
Alexander, A. J., 208
Alexander, C. C., 345
Alexander, R. D., 89, 119, 227, 228
Alfaro, A., 199
Alicata, J. E., 405
Allee, W. C., 72, 81, 162, 183, 184
Allen, H. W., 166, 294, 328
Allen, M. D., 210
Allen, O. N., 381
Allen, W. W., 167
Allred, A. M., 326
Almeida, P. R. De, 379
Altmann, G., 53
Amin, El S., 42, 43
Ammon, R., 52
Anders, F., 142, 143, 145
Andersen, A. L., 367
Anderson, A. D., 304
Anderson, D. B., 186
Anderson, E., 82
Anderson, H. W., 381
Anderson, L. D., 373, 378, 380
Anderson, N. H., 354
Anderson, R. F., 324
Anderson, T. J., 108, 111
Andison, H., 352
Andres, L. A., 327, 380
Andrewartha, H. G., 162, 165, 183, 184, 186, 195, 196, 235, 236, 240, 245, 251
Andrews, J. M., 402
Angermann, H., 209
Anglas, J., 12
Angus, T. A., 279, 283, 286, 287, 289, 292
Antongiovanni, E., 348
Apple, J. W., 372
Aragão, H. de B., 403
Arant, C. H., 379
Arant, F. S., 378, 379
Araujo, R. L., 127
Arbuthnot, K. D., 199, 282, 283, 286
Archibald, H. M., 428
Armitage, H. M., 236
Armstrong, T., 345, 355
Arnason, A. P., 371
Arnold, C. H., 346
Arnold, E. W., 372
Arnold, J. W., 3, 5, 6
Arnott, D. A., 260
Arrow, G. J., 121
Arthur, D. R., 236
Aruga, H., 68
Arvy, L., 2, 3, 4, 6, 20, 33
Ashdown, D., 378, 379
Ashman, H. G. W., see Williams-Ashman, H. G.
Asperen, K. van, 29, 311, 314
Atkins, W. G., 324
Atwood, C. E., 94
Aub, J. C., 26
Aubin, M., 187
Auclair, J. L., 141, 143, 145
Auer, C., 278, 289
Augustinnson, K. B., 311
Aurivillius, C., 100, 101
Aust, S. R., see Roegner-Aust, S.
Avens, A. W., 351
Avi-Dor, Y., 304
Axelrod, D. I., 100
Ayontantis, A. J., 120
Ayre, G. L., 354
Ayurakitkosol, L., 424, 429

B

Babcock, K. W., 199
Baccetti, B., 10
Back, E. A., 110
Bacon, R., 392
Bacot, A. W., 395
Badger, L. F., 400, 401
Baerends, G. P., 207-34; 207, 208, 212, 213, 218, 219, 221, 222, 223, 224, 225
Baggiolini, M., 345
Bailey, V. A., 240, 243, 245
Baird, R. B., 278, 280, 289
Baker, A. C., 402
Baker, C. F., 391
Baker, E. W., 354
Baker, W. C., 324
Balch, R. E., 293
Balduf, W. V., 128
Ball, G. H., 22, 24, 25, 26, 29, 30, 31, 32, 34, 162
Banerjee, S. N., 379, 380
Banks, C. J., 142, 144, 145, 147, 155
Barkmeier, H., 66
Barnes, H. F., 93
Barnes, J. M., 303, 306, 313
Barnes, M. M., 343-62; 327, 344, 347, 352, 354
Barnes, O. L., 140
Barnes, S., 321
Barsa, M. C., 25, 29, 304
Barth, R., 43
Bartlett, B. R., 328
Bartlett, M. S., 235, 247
Bastock, M., 216, 219
Basu, A. C., 379, 380
Bates, H. W., 123, 124, 125
Bates, M., 93
Batzer, H. O., 193
Baumgartner, W. J., 19
Beal, F. E. L., 128
Beament, J. W. L., 310
Beard, R. L., 9, 10, 179
Becht, G., 305, 309
Beckel, W. E., 24, 25, 32, 33
Becker, E., 66, 69, 70
Becker, G., 102, 104, 118
Becker, H., 353
Beckham, C. M., 349
Beckmann, R., 70, 71, 72
Beebe, W., 208, 221
Beeson, C. F. C., 99, 101, 102, 106, 109, 110, 112, 113, 114, 115, 116, 117, 118, 119, 129
Béguin, S., 280, 283, 287, 290
Beirne, B. P., 236
Bek, J. J. M., see Mansour-Bek, J. J.
Bělař, K., 19, 33
Belehradek, J., 185
Beljakova, M. B., 352
Bell, C. R., 280, 283
Bell, J. F., 398
Bender, E., 349
Benjamin, D. M., 193, 278, 290
Bennett, B. L., 400
Benson, R. B., 94
Benwitz, G., 2
Bequaert, J., 129
Beran, F., 323

Berger, N. E., 315
Bergold, G. H., 277, 278, 284, 285, 288, 289, 292, 293
Berlese, A., 1, 11
Berti, A. L., 422, 423, 429
Bertram, D. W., 423, 426
Bess, H. A., 170, 265, 268
Bethe, A., 39
Bettini, S., 314
Beutenmuller, W., 102
Bews, J. W., 259
Bhalla, B. D., 423, 428
Bhatia, B. M., 99, 101, 102, 106, 109, 110, 112, 113, 114, 115, 116, 117, 118, 119
Bhatia, M. L., 422, 423, 424, 425, 426, 427, 428, 429
Bhattacharyya, P. K., 381
Bieckert, E., 70, 71, 72
Bier, K. H., 49, 222, 225
Bigger, J. H., 372
Biliotti, E., 278, 279, 280, 289, 294
Birch, A. J., 62
Birch, L. C., 78, 183, 184, 186, 195, 196, 235, 236, 240, 245, 251
Bird, F. T., 277, 278, 285, 286, 287, 290, 292, 293, 295
Bird, L. S., 381
Bishop, M. W. H., 169
Bishopp, F. C., 404
Black, R. H., 423, 428, 429
Blackman, M. W., 102
Blackwelder, R. E., 101
Blair, F. 82
Blair, K. G., 102
Blanchard, R. A., 372
Blanck, A., 187
Blatchley, W. S., 120
Blest, A. D., 208, 216, 219, 224, 228
Blinn, R. C., 319, 337
Bliss, C. I., 190
Blount, B. K., 62
Blum, M. S., 310, 315
Blunck, H., 282, 283, 285, 294
Boccacci, M., 314
Boch, R., 225
Bocking, V., 322
Bodenheimer, F. S., 102, 116, 129, 139, 142, 145, 147, 151, 156, 186, 187, 190, 191, 192, 194, 196, 199, 236, 251
Bodenstein, D., 27
Bodin, N. O., 220
Boerema, L. K., 209, 219
Bolwig, N., 225
Bondar, G., 120
Bonnefoi, A., 280, 287, 290
Bonnemaison, L., 145, 147, 378
Booth, C. O., 147, 151, 152, 153
Boppe, P., 126
Bordas, E., 422, 427
Borden, A. D., 349
Börner, C., 139, 140, 142, 144, 145, 147, 155
Bosc, J. M., 101, 109, 111, 120
Bovey, P., 278, 279, 294, 351
Boving, A. G., 128
Bowling, C. C., 378
Box, H. E., 111
Boyd, M. F., 415, 417, 418, 419, 420, 422, 423, 424, 425, 426, 427, 428, 429
Bradbury, F. R., 378
Bradford, S., 26
Bradley, J. D., 349
Brase, K. D., 350
Breeland, S. G., 379
Breinl, A., 405
Breland, O. P., 89
Breny, R., 278
Breuning, S., 99, 122, 123, 125, 126
Brian, M. V., 210, 235
Brian, P. W., 381
Brickenstein, C., 212
Bridges, C. B., 429
Briggs, J. B., 349, 352
Brill, N. E., 399
Brindley, T. A., 282, 283, 286
Broadbent, L., 145, 146, 147, 150
Bronskill, J. F., 165
Bronson, T. E., 378
Brooks, A. R., 78
Brooks, F. E., 110, 128
Brooks, J. W., 373
Brooks, M. A., 144
Broquist, H. P., 53
Brown, A. W. A., 304, 309, 313
Brown, B. E., 304
Brown, B. R., 63
Brown, F. C., 210, 215
Brown, F. M., 95
Brown, R. V., 313
Brown, R. Z., 210, 215
Brown, W. J., 77-98; 80, 90, 92
Brown, W. L., 85
Bruce-Chwatt, L. J., 428
Bruch, C., 111, 120, 126, 127
Brues, A. M., 26
Brues, C. T., 128, 251, 254
Bry, R. E., 328
Bryant, J. C., 31
Bryden, J. W., 169
Bryson, H. R., 378
Buchanan, L. L., 87, 90
Bucher, G. E., 185, 278, 280, 284
Buchholtz, C., 208, 209, 217, 221, 223
Buchka, E., 120
Buchner, P., 103
Buck, J. B., 21, 25, 26, 29
Buckell, E. R., 128
Buckle, P., 162
Bucklin, D., 33
Bugbee, R. E., 259
Bugnion, E., 121, 126
Bull, L. B., 403
Burchfield, H. P., 344, 335, 336
Burgerjon, A., 289
Burges, H. D., 184, 190, 193, 200
Burgess, L., 188
Burke, H. E., 103
Burkhardt, C. C., 378
Burnes, M. L., 26
Burnett, R. G., 23
Burnett, T., 235-50; 168, 239, 241, 246
Burt, P. E., 325, 328
Burton, L., 23, 29, 34
Burton, R. K., 381
Burton, V. E., 327
Bushland, R. C., 320, 323, 332
Busnel, M., 221
Busnel, R., 221
Busnel, R. G., 68
Busvine, J. R., 303, 307, 320, 321, 322
Butenandt, A., 39-58; 40, 41, 42, 45, 52, 70, 71, 72,
Butler, C. G., 45, 46, 47, 166, 208, 210, 225
Butler, L. I., 328
Butovitsch, H. von, 115, 117, 119
Butz, E. L., 349, 353
Buxton, P. A., 186
Buxton, S. S., 378

C

Cable, R. M., 185
Calderbank, A., 63
Caldwell, N. E. H., 346
Cali, C. T., 304
Callan, I. W. M., 371
Callaway, S., 338
Callenbach, J. A., 378
Cameron, E., 257, 258, 259
Cameron, G., 17, 30, 32
Cameron, J. W. M., 185, 278
Cameron, M. L., 144
Campbell, F. L., 331
Campbell, T. G., 262, 270, 271, 272
Campos, F., 121
Canning, E. U., 284
Carlisle, D. B., 47
Carlson, E. C., 364, 366, 367, 368, 369, 371,

372, 373
Carlson, F. W., 345
Carlson, J. G., 20, 23, 33
Carpenter, S. J., 94, 423, 424, 429
Carson, H. L., 93
Carson, N. B., 323
Carter, H. E., 381
Carter, H. J., 122
Carter, R. H., 330, 331, 333
Cashmore, A. B., 262, 270, 271, 272
Casida, J. E., 310
Cassil, C. C., 345, 346
Castaneda, M. R., 400, 401
Castro, A. R., see Ruiz Castro, A.
Cats, V. de M.-M., see Meester-Manger Cats, V. de
Causey, O. R., 422, 423, 425
Cayrol, R., 279, 292
Ceder, E. T., 400, 401
Chambers, R., 18
Champion, H. C., 111
Champlain, A. B., 118, 128
Chan, F. L., 66
Chandler, S. C., 347, 351
Chang, C. S., 307, 310
Chang, S. C., 110
Chant, D. A., 354, 357
Chao, S. T., 378
Chapman, P. J., 354, 355
Chapman, R. N., 184
Charin, N., 19
Chastang, S., 27, 29, 30, 31
Chater, E. H., 259, 269
Chatters, R. M., 142, 146
Chaudhuri, R. P., 170
Chaudry, G., 190, 201
Chauvin, R., 47, 52, 65, 69
Cheesman, E., 119
Chefurka, W., 311, 315
Chemsak, J. A., 118
Chen, C. S., see Shu-Chen, C.
Cherney, L. S., 407
Cheron, G., 117
Chevrolat, L. A. A., 123
Chibnall, A. C., 152
Childs, A. F., 313
Childs, L., 346
Chinn, S. H. F., 381
Chisholm, R. D., 324
Chittenden, F. H., 111, 128
Chow, C. Y., 426
Christie, M., 420
Chwatt, L. J. B., see Bruce-Chwatt, L. J.
Ciuca, M., 422, 424, 428
Clancy, C. W., 66
Clancy, D. W., 348
Clark, E. C., 278, 285, 290, 292, 295
Clark, E. L., 379
Clark, E. W., 25
Clark, L. R., 266
Clark, N., 266
Clausen, C. P., 110, 162, 167, 168, 179, 236
Clay, T., 408
Cleare, L. D., 111
Clements, F. G., 201
Cleveland, M. L., 348
Cloudsley-Thompson, J. L., 185, 186, 187, 188, 235
Colburne, M. J., 426
Cole, L. C., 235, 251
Coles, L. W., 322, 329
Colhoun, E. H., 306, 310, 312, 314
Colless, D. H., 423, 428
Collier, W. A., 18
Collins, C. L., 186
Collins, D. L., 321, 345
Collins, W. E., 322
Collyer, E., 357
Concha y Venegas, J. A., 422, 423, 424, 426
Cong-Tiêu, N.-, see Ngugên-Cong-Tiêu
Connola, D. P., 325
Cook, C. W., 259
Cook, W. C., 187, 195, 197, 198
Cordner, H. B., 378, 379
Cordon, F. M., 142
Corella, L. B., 110
Corrin, W. R., 366, 367, 373
Costa Lima, A. da, 109, 111, 125, 392
Cottier, W., 110
Cotton, R. T., 324
Couturier, A., 291
Covell, G., 423, 424
Cowan, C. B., Jr., 379
Cowan, T. E., 372
Cowez, S., 20, 28
Cox, C. E., 373
Craighead, F. C., 79, 95, 99, 102, 103, 109, 110, 129, 193
Cralley, E. M., 380
Crane, J., 209, 210, 217, 220, 222, 224, 226, 227
Crawshay, G. A., 106, 129
Crenshaw, J. W., 95
Cressman, A. W., 185
Criddle, N., 92
Cromartie, R. I. T., 59-76; 63, 64, 72, 73
Crow, J. F., 322
Crowdy, S. H., 381
Crowell, R. L., 325, 326, 327
Cuénot, L., 11
Culpepper, G. H., 323
Cuming, F. G., 349
Cunningham, W. J., 235, 246
Currie, G. A., 251, 259, 265, 270
Curtis, O. F., Jr., 354
Cushman, R. A., 128
Cutkomp, L. K., 280, 283, 286, 289, 326
Cutright, C. R., 346, 347, 351, 355
Czihak, G., 9, 12

D

Daanje, A., 207
da Costa Lima, A., see Costa Lima, A. da
da Fonseca, J. P., see Fonseca, J. P. da
Dahm, P. A., 303, 321, 324, 331
Dameron, W. H., 253, 259
Dana, R. H., 269
Daneel, R., 68
Daniels, N. E., 378
Darlington, P. J., 99, 126
Darwin, C., 79, 80
da Silveira, M. M., see Silveira, M. M. da
Davatchi, A.G., 155
Davey, P. M., 141
David, A. L., 263
David, J. L., see Limon-David, J.
David, W. A. L., 363, 378, 379, 380
Davidow, B., 323, 336, 337, 338
Davidson, G., 418, 419, 427, 429
Davidson, J., 151, 185, 189, 192, 195
Davies, D. R., 313
Davies, R. G., 351
Davies, W. M., 269
Davis, A. C., 373
Davis, C. J., 261, 269, 272
Davis, J. W., 379
Day, M. F., 17-38; 141, 142, 403
De, R. K., 280
Dean, F. P., 345, 346, 347, 350, 355
Dean, R. W., 321, 353
Deane, L. M., 422, 423, 425
Deane, M. P., 422, 423, 425
DeBach, P., 178, 184, 236, 238, 244, 245
Decker, G. C., 186, 323, 327
de Groot, A. P., see Groot, A. P. de
De Jong, D. J., 349
Deleurance, E. P., 210, 211, 214
Demal, J., 23, 29, 33
de Martonne, E., see Martonne, D. de
de Meester-Manger Cats,

V., see Meester-Manger Cats, V. de
de Meillon, B., see Meillon, B. de
de Meira, M. T. V., see Meira, M. T. V. de
Den Boer, D. J., 354
Dennell, R., 5, 13, 73
Dennys, A. A., 345
de Peyerimhoff, P., see Peyerimhoff, P. de
der Kloot, W. G. Van, see Van der Kloot, W. G.
Derksen, W., 102
de Ruiter, L., see Ruiter, L. de
Deschamps, P., 103
Deshpande, V. G., 110
Dethier, V. G., 44, 169, 218, 222, 223, 224, 226, 228, 254, 255, 344, 345
Dever, D. A., 351
De Vincentiis, M., 68
de Vrie, M. van, see Vrie, M. van de
Dewar, S. C., 423, 428
Dewey, J. E., 348
Dezeeuw, D. J., 367
de Zulueta, J., see Zulueta, J. de
Dhir, S. L., 423, 424, 425
Dias, E., 404
Dickens, F., 315
Dicker, G. H. L., 346, 349, 351, 352
Dickinson, B. C., 345
Dickson, R. C., 344
Dill, L. E., 373
Dimroth, O., 62
Din, M. Q.-ud-, see Qutub-ud-Din, M.
Dirks, C. O., 352
Distant, W. L., 129
Ditman, L. P., 333, 373
Dixon, A. F. G., 144
Dobson, J. W., 89
Dobzhansky, T., 77, 81, 83
Dodd, A. P., 112, 252, 253, 255, 256, 257, 260, 261, 262, 263, 271
Dogger, J. R., 372
Domenichini, G., 291
Doneen, L. D., 367
Doner, M. W., 321
Donovan, F. W., 62
Dor, Y. A., see Avi-Dor, Y.
Dosse, G., 354
Douglass, J. R., 378
Doutt, R. L., 161-82; 175, 179
Dove, W. E., 401
Dowden, P. B., 278
Dowling, M. A. C., 427
Downe, A. E. R., 95
Downes, J. A., 111, 210
Downes, W., 352
Downing, R. S., 355
Downs, W. G., 422, 427
Draafsell, E. J., see Jeager-Draafsel, E.
Draper, C. C., 418, 419, 423, 429
Drees, O., 210, 218, 219, 221, 223, 224
Dreiss, J. M., 334
Dresden, D., 305, 315, 322
Dresner, E., 283
Driggers, B. F., 348
Drilhon, A., 68
Drooz, A. T., 278, 290
Drysdale, S., 306
Dudich, E., 118
Dudley, J. E., Jr., 378
Duewell, H., 62, 63
Duffield, P. C., 372
Duffy, E. A. J., 99, 107, 108, 109, 110, 111, 116, 117, 119, 120, 125, 128, 129
Dufour, G., 423, 428
Dumbleton, L. J., 110
Dumortier, B., 221
Dunbar, R. W., 93
Dunn, J. A., 145
Dunn, P. H., 277, 280, 281, 283, 284, 285, 290, 291, 292, 293, 295
Durasova, M., 344
Dürr, H. J. R., 109, 114, 117, 130
Durrant, S. D., 86
Duryee, W. R., 20, 33
Dusham, E. H., 117, 128
Dustan, A. G., 373
Dutky, S. R., 279, 282, 283, 288, 289, 291, 292, 293
Dutt, N., 120
Dy, F. J., 422, 423, 424 425, 426, 427, 428, 429
Dye, D. W., 381
Dyer, R. E., 399, 400, 401, 402

E

Eagle, H., 27
Earle, W. R., 22, 25, 31
Eastop, V. F., 151
Eaton, C. B., 109
Eaton, J. K., 351
Eddy, G. W., 320, 323, 334
Eden, W. C., 378
Edwards, R. L., 219, 245
Egelhaaf, A., 52, 66, 67
Ehrlich, H., 210
Eidmann, H., 102
Ejercito, A., 424, 428
Ekblom, T., 103
Elens, A. A., 192
Elgee, D. E., 286, 290, 292, 293, 295
Elliott, E. A., 128
Ellis, H. W., 116
Elmore, J. C., 373
el Shatoury, H. H., see Shatoury, H. H. el
El-Ziady, S., 142, 147, 155
Emden, F. I. van, 109, 128
Emerson, A. E., 77, 81, 162, 184, 210, 227
Emerson, R. B., 379
Engelmann, F., 48
Ergle, D. R., 381
Ermin, R., 2, 6
Erwin, D. C., 366, 369
Erwin, W. R., 330, 331
Esch, I. van, 29
Escherich, K., 109
Eschirch, B., 68
Eskey, C. R., 399
Essig, E. O., 183, 254
Eugster, C. H., 61
Eulitz, F., 66
Evans, A. C., 141
Evans, C. A., 398
Evans, H. E., 94, 216, 227, 228
Evans, V. G., 31
Evenhuis, H. H., 144
Evlakhova, A. A., 281
Ewing, H. E., 392
Eyer, J. R., 344, 345
Eyles, D. E., 419

F

Faber, A., 119, 221
Fahey, J. E., 346, 347, 348
Falck, R., 104
Faldini, J. D., 280
Farid, M. A., 424, 426, 427, 428, 429
Faust, E. C., 407, 420, 422, 423, 424, 425, 426, 427, 428, 429
Fawcett, H. S., 183
Fay, R. W., 325, 326, 327
Feder, N., 33
Felt, E. P., 103, 109
Feng, L. C., 426
Fernald, H. T., 195, 371
Ferris, G. F., 78
Fester, G. A., 62
Fick, R., 62
Fidler, J. H., 374
Fidler, W. E., 68
Filmer, R. S., 372
Finlayson, D. G., 366, 373, 374
Finlayson, L. H., 224
Finney, G. L., 166
Fiori, B., 347
Fischer, I., 20, 28
Fischer, L., 424, 425
Fisher, F. E., 281, 292
Fisher, R. W., 312, 333
Fisher, T. W., 184, 236, 284
Fisher, W. S., 101, 111
Fiske, W. F., 127, 128

Fitzgibbon, M., 366
Fitzhugh, O. G., 333
Fitz-James, P., 279, 289
Fjelddalen, J., 355
Flanders, S. E., 94, 163, 165, 166, 167, 169, 170, 171, 173, 176, 177, 178, 243, 257
Flaschenträger, B., 43
Fleck, E. E., 324
Fleming, W. E., 322, 329
Fletcher, F. W., 363
Flik, H. M., 355
Flitters, N. E., 200
Floch, H., 422, 423, 426
Flock, R. A., 327
Florey, E., 307
Florkin, M., 9, 24
Floyd, E. H., 78, 373
Fluke, C. L., 351
Fogh, T. W., see Weis-Fogh, T.
Fonseca, J. P. da, 120
Forbes, A. R., 374
Forbes, S. A., 123, 128
Ford, E. B., 65
Forgash, A. J., 315
Forrest, H. S., 52, 66, 67
Forster, W., 52
Fowler, K. S., 306, 310, 312
Fox, C., 408
Fox, C. J. S., 281
Fox, H., 187, 197
Fox, I., 392, 401
Fox, R. M., 427
Fox, W. B., 371
Fraenkel, G., 53, 143, 152, 187
Francis, E., 399
Frankenfeld, J. C., 324
Franssen, C. J. H., 111, 113
Franz, E., 122
Franz, J., 144, 278, 284, 285, 292, 293
Frawley, J. P., 333
Free, J. B., 45, 208, 210
Freeman, G. H., 354
Freeman, R. B., 408
Freeman, T. N., 79, 95
French, R. G., 380
Frew, J. G. H., 18, 23, 33
Friedman, F., 23, 29, 34
Frings, H., 209
Frings, M., 209
Frisch, K. von, 46, 210, 225
Fröbrich, G., 53
Froggatt, W. W., 110
Frontali, N., 311
Frost, M. H., Jr., 373
Frost, S. W., 344
Füchtbauer, H. S., see Swart-Füchtbauer, H.
Fukami, J., 304, 315
Fukuto, T. R., 303, 311, 313, 379
Fullaway, D. T., 112, 253, 260, 262, 265, 267, 268, 270, 271
Fuller, C., 111, 120
Fuller, F. B., 399
Fulton, B. B., 89, 227
Fulton, H. G., 374
Furman, D. P., 326
Furniss, R. L., 109
Furr, R. E., 379
Fye, R. E., 379
Fyfe, R. V., 270

G

Gabaldon, A., 422, 423, 424, 429
Gabe, M., 3, 4, 6, 20, 33
Gahan, C. J., 111, 119, 124, 125
Gahan, J. B., 325, 326
Gambrell, F. L., 350
Gambrill, R. G., 353, 354
Gañan, D., 422, 426
Ganapathipillai, A., 423, 427
Gannon, N., 327
Gardiner, B. O. C., 363, 378, 379, 380
Gardiner, L. M., 117, 118
Gardner, J. C. M., 262
Gardner, T. R., 167, 261
Garman, H., 129
Garman, P., 344, 352, 353
Garmus, R. D., 327
Garthside, S., 251, 259, 265
Gasser, R., 353, 355
Gaulden, M. E., 20, 22, 23, 28, 33
Gause, G. F., 201, 242, 248
Gauthier, J. C., 394
Gavrilov, W., 20, 28
Gayspitz, K. F., 164
Geier, P., 349
Gelfand, H. M., 423, 427
Gerberg, E. J., 115
Gerhardt, R. W., 283
Gerok, W., 41
Gerould, J. H., 82
Gersdorf, W. A., 330, 331
Ghulamullah, 238
Giannotti, O., 309
Gibbons, D. A., 47
Gibson, F. J., 95
Gilbert, I. H., 326
Gillies, M. T., 419, 420, 423, 429
Gilmer, R. M., 350
Ginsberg, S., 313
Girth, H. B., 278
Gjullin, C. M., 186, 187, 198
Glaser, R. W., 18, 22, 25, 33, 34, 65
Glasgow, H., 373, 374
Glass, E. H., 279, 293, 347, 348, 349
Glock, G. E., 315
Glusenkow, N. A., 346
Goette, M. B., 326
Goillot, C., 52
Gojmerac, W. L., 378
Goldberg, L., 53
Goldschmidt, R., 17, 18, 33
Gonda, O., 304
Good, N. E., 392, 402
Goodchild, A. J. P., 32
Gooden, E. L., 279
Goodwin, T. W., 60, 61, 68, 69
Gordon, H. T., 306
Gorjaceva, E. P., 352
Goryushin, V. A., 374
Gorzycki, L. J., 379
Gostick, K. G., 323
Goto, A., 327
Goto, M., 66
Gottlieb, D., 381
Gottschewski, G., 20, 28
Götz, B., 40, 43
Gough, H. C., 374
Gould, E., 345, 354
Gounelle, E., 127
Gowdey, C. W., 308
Gowen, J. W., 175, 176
Grace, T. D. C., 17-38; 21, 22, 23, 25, 26, 27, 28, 29, 30, 31, 32, 34
Graf, J. E., 194
Graham, C., 349, 353
Graham, S. A., 94, 102, 129, 183
Grahn, M., 311
Grainger, M. M., 351
Grandi, G., 110, 111, 112, 123, 128
Grandori, R., 355
Granett, P., 325
Grassé, P. P., 49, 210
Grassi, B., 49
Grassi, G. B., 406
Green, A. A., 321, 323
Green, A. L., 313
Green, G. W., 187, 188
Green, R. G., 398
Greenshields, F., 173
Greenslade, R. M., 378
Greer, E. N., 324
Grégoire, C., 9, 10
Gregory, K. F., 381
Grenelly, R. E., 381
Gressitt, J. L., 99, 100, 102, 110, 111, 112, 122, 127, 129, 183
Grewal, M. S., 404
Griffith, M. E., 424, 429
Griffiths, J. T., 7, 281, 292
Grison, P., 278, 279, 280,

283, 284, 286, 287, 289, 290
Grjebine, A., 423, 424, 425, 426, 427, 428
Groblewski, G. E., 313
Groot, A. P. de, 47
Grosch, D. S., 174
Grossbach, U., 66, 67
Grösswald, K., 104
Groves, J. R., 345, 349, 353
Günder, J. Z., see Ziegler-Günder, J.
Gunderson, H., 372
Gunn, D., 110
Gunn, D. L., 186, 187
Gunther, C. E. M., 424, 428
Gunther, F. A., 319, 337
Günther, S., 291
Gurney, A. B., 78
Guthrie, F. E., 186, 280, 292
Guy, E. I., 324

H

Haas, A., 46, 48, 208
Hackman, R. H., 26, 29, 60, 61, 65, 66, 69
Hadaway, A. B., 200
Hadorn, E., 52, 66, 67
Hadwen, S., 404
Hagen, K. S., 353
Haine, E., 148, 149, 150
Haines, T. W., 324
Halcrow, J. G., 427
Haldane, J. B. S., 225
Halfhill, J. C., 291
Haliburton, W., 103, 105
Hall, I. M., 277, 279, 280, 281, 282, 283, 284, 285, 286, 288, 290, 291, 292, 293, 295
Hall, M. J., 354
Hallemans, A., 351
Haller, H. L., 43
Hallowes, K. H., 306
Hamilton, D. W., 346, 347, 348
Hamilton, J., 120
Hamon, J., 423, 424, 425, 426, 428
Hamstead, E. O., 345, 354
Hancock, G. L. R., 128
Handford, R. H., 366, 373, 374
Hankin, E. H., 394
Hanks, J. H., 17, 32
Hanna, R. L., 379
Hannay, C. L., 279, 289
Hanser, G., 72
Haramoto, F. H., 268
Hardman, J. A., 349
Hardouin, R., 111
Harley-Mason, J., 73
Harlow, P. A., 311
Harman, S. W., 346
Harries, F. H., 185, 378, 379, 380
Harris, E., Jr., 374
Harris, G. A., 266
Harris, W. B., 350
Harrison, A., 311, 312, 313, 314
Harrison, R. G., 17
Hart, M. P., 291
Hartley, G. S., 378
Hartshorne, R., 190
Hartzell, A., 21, 33, 332, 334, 335, 336
Harvey, G. T., 313
Harville, J. P., 292
Harwood, R. F., 366, 371, 372
Hasegawa, K., 129
Haseman, L., 345
Haskell, P. T., 221
Hastings, E., 372
Hathaway, C. R., 392
Haufe, W. O., 188
Hawboldt, L. D., 349
Haydak, M. H., 51, 52, 53
Hayes, R. O., 26
Hayes, W. J., 409
Headlee, T. J., 393
Healey, M. J. R., 371
Heathcote, G. D., 151
Hebard, M., 89
Hebb, C. O., 312
Heck, S. V. H., see Von Hörmann-Heck, S.
Hecker, E., 40, 41, 42
Heimpel, A. M., 279, 280, 282, 286, 287, 288, 289
Hein, G., 225
Heintze, A., 115, 116
Heinz, H. J., 212, 226, 229
Heinze, K., 139, 140, 142, 144, 145, 147, 155, 260
Heitz, E., 103
Hellqvist, H., 374
Hellyer, G. C., 314, 315
Helson, G. A. H., 236
Henderson, C. F., 354
Henson, W. R., 188
Heran, H., 210, 225
Heriot, A. D., 345
Herman, C. M., 404
Hernandez, L. U., see Ureña Hernandez, L.
Herrick, G. W., 103, 109
Hervey, G. E. R., 279, 289, 293, 366, 373
Hess, A. D., 110, 128, 424, 428
Hesse, R., 183
Hewlett, P. S., 323
Heymann, H., 66
Hibbard, H., 19
Hijner, J. A., 142
Hilchey, J. D., 334, 335, 336
Hill, A. R., 146
Hill, C. H., 349
Hill, G. P., 141
Hille Ris Lambers, D., 152, 153, 154, 155, 156
Hills, F. J., 367
Hills, O. A., 380
Hinds, W. E., 194
Hinton, H. E., 10, 235
Hirata, Y., 66
Hiratsuka, E., 141
Hironaka, M., 266
Hirst, L. F., 409
Hoare, C. A., 404
Hobbiger, F., 313
Hodgkin, E. P., 422, 423, 424, 425, 427, 429
Hodson, A. C., 184, 185, 352
Hoffman, W. E., 110
Hofmaster, R. N., 366, 367, 373
Hogan, T. W., 346
Holbrook, D. V., 313
Holdaway, F. G., 189, 195
Holland, G. P., 391, 392
Hollande, A. C., 1, 65, 116
Holloway, J. K., 251, 258, 266, 269
Holmes, E., 356
Holmes, R., 313
Holmstedt, B., 311
Hoof, H. A. van, 142, 151
Hopkins, A. D., 106, 183, 189, 194, 199
Hopkins, A. R., 379
Hopkins, D. E., 334
Hopkins, G. H. E., 390, 392, 407
Horikawa, M., 11
Horn, O., 104
Hornstein, I., 320, 324, 328
Horsfall, W. R., 420, 422, 423, 424, 425, 426, 427, 428, 429
Horton, J. R., 110
Hosaka, E. Y., 268, 269, 270, 272
Hoskins, W. M., 320, 326, 327, 330, 331
Hough, W. S., 283, 346, 348, 349
Houlbert, C., 109
House, H. L., 165
Hovanitz, W., 82
Howe, R. W., 184, 190, 200
Howe, W. L., 366, 367, 373
Howitt, A. J., 374
Hoy, J. M., 259, 268, 285, 288, 291
Hoyle, G., 25, 311
Hoyt, S. C., 347, 348, 350, 354, 355
Hrdý, I., 4
Hsieh, H., 424, 426
Hsu, S. C., 426

Hubanks, P. E., 333
Hubbard, C. A., 392
Hubbard, H. G., 110
Hubbell, T. H., 81, 82, 83, 86, 87, 89
Huber, F., 209, 212, 214, 215, 217, 221
Hudson, G. V., 101, 102, 122
Hueck, H. J., 353, 354
Huff, C. G., 7
Huffaker, C. B., 251-76; 235, 242, 243, 251, 252, 253, 254, 256, 257, 258, 259, 266, 269
Hughes, J. H., 420
Hughes, K. M., 284
Human, J. P. E., 62, 63, 64
Humphries, K. P., 372, 378
Hungerford, H. B., 110
Hurpin, B., 291
Husain, M. A., 110
Husain, M. Z. Y., 423, 428
Hutchinson, A. H., 190
Huxley, J. S., 255
Huysmans, C. P., 351

I

Iglinsky, W., Jr., 378
Ilse, D., 220, 222
Imms, A. D., 251, 253, 254
Inagami, K., 42, 72
Ingalsbe, D. W., 332
Ingles, L. G., 102
Inhoffen, H. H., 43
Ioff, I., 405, 408, 409
Ioff, I. G., 392, 393, 402, 409
Irzykiewicz, H., 141, 142
Isakova, N. P., 288
Ishii, T., 305
Ishikura, H., 322, 327
Ito, Y., 140, 145
Ivy, E. E., 378, 379
Iwasa, T., 201
Iyengar, M. O. T., 424, 425, 427, 428
Izotova, T. E., 374

J

Jackson, C. H. N., 196
Jackson, E. B., 26
Jacobs, S. E., 280
Jacobs, W., 209, 227
James, P. F., see Fitz-James, P.
Jameson, E. W., Jr., 392
Jameson, H. R., 371
Jander, R., 210, 225
Janet, C., 1
Janisch, E., 185, 284, 292, 295
Janjua, N. A., 110
Janvier, H., 259
Jaques, R. P., 281
Jarczyk, H. J., 43
Jaynes, H. A., 167, 236
Jeager-Draafsel, E., 354
Jeffery, G. M., 419
Jellison, W. L., 389-414; 392
Jensen, V., 378
Jerrel, E. A., 279, 289
Jewett, H. H., 324
Johannsen, O. A., 371
Johannson, A. S., 11
Johansen, C. A., 328
Johansson, T. S. K., 51
Johnson, A. W., 62, 63, 64
Johnson, B., 148, 149, 150, 151
Johnson, C. G., 148, 149, 150
Johnson, D. R., 378
Johnson, G. A., 379
Johnston, C. D., 303
Jones, D. B., 52
Jones, D. P., 364, 369, 371, 372, 374, 378
Jones, E. W., 378
Jones, F. G. W., 167, 367, 372, 378
Jones, F. M., 123, 126, 128
Jones, H. A., 323, 324, 331
Jones, J. C., 2, 3, 4, 5, 9, 13
Jones, L. R., 336
Jordan, K., 392
Joshi, B. S., 63
Jourdheuil, P., 378
Joutel, L. H., 103
Jover, H., 128
Joyeux, C., 406
Jucker, E., 60
Judah, J. D., 303
Judd, W. W., 285
Junge, H., 60, 61, 68

K

Kaiser, P., 51
Kaissling, K.-E., 42
Kakizawa, H., 66
Kalf, G. F., 26
Kalmus, H., 46, 210, 211
Kalshoven, L. G. E., 110, 111, 116, 117, 123
Kämmerer, H., 62
Kannan, K. K., see Kunhi Kannan, K.
Kantack, E. J., 366, 380
Kantzes, J. G., 373
Karafiat, H., 144
Karandakar, K. R., 110
Karlson, P., 39-58; 50, 53, 69
Karrer, P., 52, 60, 61, 66, 67
Kartman, L., 326
Kaszab, Z., 119
Kawase, S., 68
Kearns, C. W., 303, 305, 306, 307, 309, 310, 315
Kearns, H. G. H., 350
Keen, F. P., 102, 103, 109
Kellogg, W. H., 396
Kelsey, J. M., 279
Kemner, N. A., 99, 111, 116
Kemp, H. K., 346
Kemper, H., 7
Kendeigh, S. C., 192
Kendrick, J. B., Jr., 367, 381
Kennedy, A. H., 406
Kennedy, J. S., 139-60; 140, 142, 143, 145, 146, 147, 148, 149, 150, 151, 152, 153, 155, 218
Kennett, C. E., 235, 242, 251, 252, 256, 266
Kenten, J., 313
Kepner, R. A., 372
Kerr, W. E., 175
Ketchel, M. M., 22, 30, 33
Kewitz, H., 313
Key, K. H. L., 89
Keys, T. E., 391
Khan, A. W., 110
Khan, M. H., 197
Kilpatrick, J. W., 324, 327
Kimmey, J. W., 109
King, H. L., 322
King, J. L., 110
King, K. M., 371, 374
King, R. C., 23
King, W. V., 332
Kirby, A. H. M., 346, 351, 353, 354
Kirk, H. B., 118
Kirnhuber, P., 338
Kitzmiller, J. B., 93, 94
Klein, A. K., 331, 338
Kleine, R., 128
Kloke, A., 329
Kloor, L. A., Jr., 371, 372
Klots, A. B., 78, 82
Knight, H. H., 65
Knipling, E. F., 323, 344
Knull, J. N., 112, 118, 125
Knuth, P., 116
Knutson, H., 366, 380
Kobayashi, H., 424, 427
Koch, A., 144
Kocher, C., 330, 338
Koehler, B., 364
Koelle, G. B., 313
Koepke, J. A., 401
Kohls, G. M., 401
Kohring, V., 19
Koide, M., 337
Koidsumi, K., 190, 199, 200, 286

Kojima, T., 99, 111, 120, 122, 129
Kokomoor, K. L., 28
Kollros, J. J., 306, 307, 310, 311
Konar, G., 280
König, M., 214
Kopech, G., 17
Köppen, W., 189
Korte, F., 66
Kortzas, C. B., 120
Kotschmar, A., 68
Kotte, W., 344
Koula, V., 344
Kovačevič, Ž., 284, 292, 295
Koyama, R., 129
Kozhanchikov, I. V., 184, 185, 186, 191
Kramer, M., 380
Kratz, F. W., 429
Krause, G., 23
Krauss, N. L. H., 261
Kremer, J., 1
Krestensen, E. R., 353
Krieg, A., 278, 280, 284, 285, 287, 289, 292, 293
Krijgsman, B. J., 315
Kriner, E. R., 348
Krishnamurthy, B. S., 423, 427
Krishnan, K. S., 422, 423, 424, 427
Krishnaswami, A. K., 425, 427
Krogerus, R., 102
Krüger, E., 208
Kubota, K., 199
Kudrina, M. A., 374
Kuenen, D. J., 352, 353, 354
Kugler, H., 221, 224
Kühn, A., 52, 66, 67
Kuhn, R., 61
Kulash, W. M., 324, 372
Kullenberg, B., 44, 46, 217, 225
Kunhi Kannan, K., 110
Kunkel, A. M., 313
Kunze, F. M., 331, 338
Kuroda, Y., 23, 33
Kyao, I. I., 164

L

Laarman, J. J., 219, 224
La Casse, W. J., 94, 423, 424, 429
Lack, D., 245
Lagrange, E., 393
Laidlaw, H. H., Jr., 175
Laing, J., 167
Laird, M., 428
Lal, R., 145
Lamb, K. P., 143
Lambers, D. H. R., see Hille Ris Lambers, D.
Lameere, A., 100, 121, 123, 126
Landi, J., 184, 236, 238
Landis, B. J., 194
Lane, F., 120, 122, 125
Lane, M. C., 363, 364, 371
Lange, A. W., 266
Lange, H. H., 3
Lange, R., 49, 52
Lange, W. H., 363-88; 349, 363, 364, 366, 367, 368, 369, 371, 372, 373, 378, 380
Languillon, J., 423, 424, 426
Lapied, M., 293
La Plante, A. H., 352
Larchenko, K. I., 1
Lartschenko, K., 10
Larsen, E. B., 192
Larson, C. L., 398
Larson, J. D., 278, 290
Lathrop, F. H., 186, 344, 352
Laug, E. P., 323, 330, 331, 333, 338
Laugenbuch, R., 285
Lavoipierre, M., 53
Law, R. G., 406
Layne, G. W., Jr., 350
Lazarencko, T., 1, 2, 10, 18, 33
Lea, A. M., 110, 125
Leach, L. D., 364, 366, 367, 368, 369, 371, 372, 373
Leake, C. D., 34
Lecompte, J., 45, 46, 208, 210, 219, 221, 224
LeConte, J. L., 116, 118
Lederer, E., 60, 66, 68
Lederer, G., 220
Leech, H. B., 109, 120
Lees, A. D., 140, 147, 164 165, 353
Lefroy, H. M., see Maxwell-Lefroy, H.
Legay, J. M., 141
Legge, J. W., 69
Lehrman, D. S., 207
Leighly, J., 184, 186, 188, 195, 198, 236
Le Masne, G., 210
Lembach, J. V., 325
Lemberg, R., 69
Lemoigne, M., 280, 287, 290
Lepesme, P., 110, 111, 112, 119, 122, 123, 125, 128
Lesne, P., 111
Lewis, D. J., 423, 424, 429
Lewis, M. R., 18, 25, 28, 33
Lewis, S. E., 303-18; 306, 310, 311, 312
Lex, T., 225
Lexow, S., 62
L'Helias, C., 2
Lhoste, J., 3, 4, 6
Liebman, E., 6
Liebster, G., 351
Lienk, S. E., 354, 355
Lieu, K. O. V., 110
Light, S. F., 49, 50
Likely, G. D., 22, 25
Lilly, J. H., 363, 372, 373
Lima, A. da C., see Costa Lima, A. da
Lin, S., 184, 185
Lindauer, M., 210, 225
Lindemann, C., 143, 145
Linden, M. von, 69, 70
Lindgren, D. L., 200
Lindquist, A. W., 323, 324, 325, 326
Lindroth, C. H., 99, 189
Lindsay, D. R., 324
Linell, M. L., 102, 111
Link, V. B., 396, 397, 402
Linsley, E. G., 99-138; v, 77, 84, 88, 95, 99, 100, 101, 103, 105, 108, 109, 118, 120, 124, 126, 327
Linzen, B., 72
Lipa, J. J., 283, 285, 294
Lipke, H., 53, 143, 152
Liston, W. G., 395
Liu, C. Y., 392
Lloyd, D. C., 168, 272
Lloyd, E. P., 379
Lloyd, N. C., 351
Locker, B., 392
Lockley, R. M., 403
Loeb, M. J., 21, 24, 25, 27, 30, 32, 33
Loeser, E., 52, 66, 67
Lohmeyer, V. K., 347, 348
Long, W. H., 372
Lopez-Neyra, C. R., 406
Lord, F. T., 237
Lord, K. A., 313
Loschiavo, S. R., 225
Lotka, A. J., 416
Lotmar, R., 12
Loughheed, T. C., 24, 26
Louis-Marie, Père, 260
Lovas, B., 287, 289
Lovell, J. H., 116
Lower, H. F., 285
Lucas, C. C., 52
Ludwick, C. F., 323
Ludwig, C., 185
Ludwig, D., 26, 185, 186, 195, 304, 321
Lukasiak, J., 423, 428
Lukoschus, F., 53
Lukyanovich, F. K., 184
Lundberg, S., 109
Lundgren, L., 219
Lunsford, C. J., 406
Lüscher, M., 20, 24, 33,

49, 50, 51, 210, 225
Lutz, F. E., 89
Lyche, H. T., see Tambs-Lyche, H.
Lyon, R. L., 109
Lysenko, V. F., 423, 424, 429

M

McAlister, H. V., 347
McAlister, L. C., Jr., 323
McAllan, J. W., 144
McArthur, J., 423, 428
McBurnie, H. V., 354
McCabe, P. J., 277, 279, 290
Macchiavello, A., 409
McClanahan, R. J., 373, 374
McConnell, E., 280, 283, 286, 289
McCoy, G. W., 398
Macdonald, G., 415, 416, 418, 419, 422, 424
Macdonald, S. F., 62, 63, 64
McDuffie, W. C., 326
McEwen, F. L., 279, 289, 293, 366, 373
Macgillivray, J. H., 367
McGregor, I. A., 423, 426
McGregor, W. S., 334
Machado, W., 345
Machay, M. L., 282, 283, 284, 287, 289
McIvor, B. C., 407
McKay, M. A., 306, 312, 313, 314, 315
McKeown, K. C., 101, 102, 117
McKerras, M. J., 425
McKinlay, K. S., 351
McLean, D. M., 380
MacLellan, C. R., 349
MacLeod, D. M., 129, 281, 282, 285, 287, 291
McLeod, J. A., 406
McLeod, J. H., 239
McMahon, M. C., 398
McPhail, M., 190
MacPhee, A. W., 237
MacSwain, J. W., 109, 327
Madden, A. H., 323, 324
Madsen, H., 408
Madsen, H. F., 347, 348, 349, 350, 354, 355
Maehler, K. L., 259
Magnus, D., 43, 209, 220
Maier, P. P., 324
Mail, G. A., 187
Mailloux, M., 349
Maines, W. M., 322, 329
Mainland, G. B., 81, 84
Majumder, S. K., 280, 285, 287
Makino, K., 42
Maltais, J. B., 142
Manadhar, T. L., 423, 428
Manger Cats, V. de M.-, see Meester-Manger Cats, V. de
Mani, M. S. 110
Mann, H. D., 333
Manning, A., 216, 224, 225
Mansfeld, K., 189, 197
Manson, G. F., 371
Mansour, K., 103, 104
Mansour-Bek, J. J., 103, 104
Manunta, C., 60, 61, 64, 65
Manwell, R. D., 415, 417, 418, 419, 422
Maple, J. D., 179
Marais, S. J. S., 264
Maramorosch, K., 32, 34
March, R. B., 304, 309, 311, 379
Marcovitch, S., 145
Marcu, O., 118, 119
Marcus, P. I., 21, 25
Marcuzzi, G., 196
Marie, Père L., see Louis-Marie, Père
Markkula, M., 140, 145, 147
Marshall, J., 346, 357
Martignoni, M. E., 32, 278, 279, 284, 286, 289, 294, 295
Martin, C. H., 185
Martin, C. J., 395
Martin, H., 351
Martonne, E. de, 189
Martouret, D., 279, 280, 287, 290
Masaitis, A. I., 371
Masera, E., 288, 294
Mason, H. S., 73
Mason, J. H., see Harley-Mason, J.
Massee, A. M., 344, 350, 351, 356
Mastbaum, O. J., 423, 429
Mathew, M. I., 424, 427
Mathis, W., 324
Matsuura, S., 66
Matthes, E., 218
Matthewman, W. G., 373
Mattson, E. L., 379
Maxcy, K. F., 400
Maxon, M. G., 311, 379
Maxwell, T. A., 407
Maxwell-Lefroy, H., 110
May, A. W. S., 111, 347
Mayer, G., 212
Mayer, H., 209, 219
Mayet, V., 110
Mayr, E., 77, 79, 81, 83, 84, 85, 86, 88, 95, 106
Meadley, G. R. W., 266
Meester-Manger Cats, V. de, 140
Meeuse, B. J. D., 209, 219
Meffert, R. L., 345
Mehra, R. N., 110
Meillon, B. de, 53, 423, 424, 425, 426, 427, 428, 429
Meira, M. T. V. de, 426
Meislich, E. K., 313
Melampy, R. M., 52
Melander, A., 356
Mellanby, K., 130
Melzer, J., 122
Mendizabal, M., 110
Menon, K. P. V., 281, 287, 291
Menon, M. A. U., 424, 427
Merkel, M. E., 379
Merrell, D. J., 322
Merriam, C. H., 189, 191
Merrill, L. G., 367
Merritt, J. M., 381
Messenger, P. S., 183-206; 200, 327
Metcalf, R. L., 68, 69, 303, 304, 309, 311, 325, 379
Metselaar, D., 422, 423, 424, 425, 427, 428, 429
Meuchel, J., 266
Meyer, A., 189
Meyer, K. F., 397
Michelbacher, A. E., 184, 188, 195, 198, 236
Micks, D. W., 95
Millara, P., 20
Miller, D., 253, 254, 255, 257, 259, 266, 268, 269, 270, 271
Miller, D. D., 94
Miller, G. L., 44
Miller, J. M., 109
Miller, L. A., 373, 374
Miller, N. C. E., 111
Milliron, H. E., 239
Mills, H. B., 184
Mills, W. D., 352
Milne, A., 144, 235, 236, 251
Milum, V. G., 225
Misra, B. G., 424, 427
Mitchell, H. K., 52, 66, 67
Mitchell, L. C., 331, 338
Mitchell, W. C., 373
Mittelstaedt, H., 212
Mittler, T. E., 140, 141, 142, 143, 145, 147
Miyajima, S., 337
Mjoeberg, E., 117
Moericke, V., 148, 149, 150, 151
Moise, R., 429
Moore, J. A., 84
Moore, N. W., 208
Mooser, H., 399, 400, 401
Mordvilko, A. K., 140, 152, 154
Morgan, A. C., 111
Morgan, C. V. G., 117, 354, 357

Morgan, J. F., 24, 30
Morgenthaler, P. W., 5
Morley, C., 128
Morris, M., 266
Morris, M. G., 324
Morris, R. F., 236
Morrison, F. O., 321
Morrison, H. E., 371
Morrison, P. E., 304, 309
Morton, H. J., 24
Moshman, J., 22
Mostafa, K. A., 379
Mouchet, J., 423, 424, 426
Mount, R. A., 422, 429
Muesebeck, C. F. W., 128
Muir, F., 251
Muir, R. C., 354
Muirhead-Thomson, R. C., 423, 424, 425, 428
Muldrew, J. A., 7, 170
Mules, M. W., 403
Müller, G., 66
Müller, H. J., 80, 145, 149, 150, 151
Müller, W., 104
Mullins, L. J., 306, 307, 309
Muma, M. H., 281, 292
Mune, T. L., 253, 261, 262
Munger, F., 185
Munroe, R. J., 372
Munson, S. C., 2, 11, 12
Murray, M. R., 17, 18
Murthi, B. K., 110
Müssbichler, A., 47
Muthu, M., 280, 285, 287
Muthukrishnan, T. S., 263
Myers, I., 52

N

Nachmansohn, D., 312, 313
Nagasawa, S., 319-42
Naidu, M. B., 315
Nakajima, M., 324
Naqvi, S. H., 423, 428
Narahashi, T., 305, 309
Narayanan, E. S., 170, 263
Nardy, R. V., 321
Nash, R., 322
Nash, T. A. M., 190, 196
Natti, J. J., 366, 367, 373
Naude, T. J., 264
Nauk, E. G., 34
Nawa, S., 66, 68
Neander, A., 117
Neill, M. H., 400
Neklesova, I. D., 374
Nelson, R. H., 324, 330, 331
Nelson, W. L., 366, 371, 372
Neubert, G., 70
Neururer, J., 323
Newcomer, E. J., 344, 345, 346, 347, 350, 355
Newlin, O., 367, 369
Newman, J. F., 330, 332, 339
Newsom, L. D., 78, 379
Newton, W. L., 405
Neyra, C. R. L., see Lopez-Neyra, C. R.
Nguyên-Cong-Tiêu, 110
Nicholson, A. J., 126, 235, 236, 241, 243, 245, 247, 251, 253
Niegisch, A. D., 45
Nielsen, A. T., 210
Nielsen, E. T., 210, 228
Nielson, M. M., 283, 286
Nielson, M. W., 140
Niesiolowski, W., 117
Niklas, O. F., 278, 279, 285
Nirula, K. K., 281, 287, 291
Nix, M., 22
Nixon, H. L., 142, 144, 147
Noiret, C., 49
Nolan, K., 330, 331
Noll, J., 185
Nomura, K., 189
Norton, L. B., 324, 348
Nugent, T. J., 366, 367, 373
Nuorteva, P., 142, 144
Nuttall, G. H. F., 394

O

Oberholzer, R. J. H., 215
O'Brien, R. D., 303, 311, 314
Ochsé, W., 10
O'Connor, B. A., 253, 261, 262
Odlund, M. L., 373
Oertel, E., 1
Ogata, M., 394
Ogden, D. B., 378
Ögel, S., 4
Ohuye, T., 11
Oka, I. N., 280
Okay, S., 61, 65, 69
Oku, M., 60
Olarte, J., 399
Olliff, A. S., 110
O'Loughlin, G. T., 347
Olsufiev, H. G., 398
Oman, P. W., 90
Oppenoorth, F. J., 322
Osborn, H. T., 259, 352
Ossowski, L. L. J., 279, 287, 290, 291
Oudemans, A. C., 392
Ozaki, K., 322, 327

P

Paillot, A., 120
Pain, J., 47, 49, 225
Paine, R. W., 267
Painter, H. R., 128
Painter, R. H., 142, 145
Pal, R., 324, 325, 422, 423, 424, 425, 426, 427, 428, 429
Palacios, M., 422, 424, 427
Palliot, A., 18
Palm, C. E., v, 346
Palm, N. B., 10
Palm, T., 102, 109
Palmer, L. S., 65
Pankaskie, J. E., 321, 331, 334
Paradis, R. O., 351
Paramanow, S., 111
Parki, L., 210
Parencia, C. R., Jr., 379
Parham, J. W., 253, 261, 262
Park, O., 77, 81, 112, 162, 184
Park, O. W., 175, 176
Park, T., 77, 81, 162, 184, 248
Parker, H. L., 166, 236, 372
Parker, R. C., 17, 24, 30, 32
Parkin, C. A., 321, 323
Parkin, E. A., 103, 104, 105, 363
Parrish, C. P., 378
Parsons, W. T., 266
Passager, P., 424
Pastrana, J. A., 280
Patch, E. M., 371
Pathak, M. D., 142
Patterson, J. T., 82, 83, 93
Patterson, N. A., 349
Paulian, R., 102, 128
Paullin, J. E., 400
Payne, M. A., 19
Payne, N. M., 238
Peairs, L. M., 191
Pechuman, L. L., 110
Peck, O., 94
Pedersen, M. W., 259
Peel, A., 141
Peffly, R. L., 326
Pelecassis, E. D., 120
Pepper, J. H., 190, 194
Perdeck, A. C., 209
Pérez, C., 6
Perez, M. Q., see Quilis Perez, M.
Perkins, R. C. L., 254, 255, 258, 260, 261
Perttunnen, V., 2
Pesqueira, M. E., 422, 427
Pesson, P., 5, 13
Peters, D. C., 142
Peters, H. M., 212, 227
Peters, W., 423, 424, 427, 428
Petersen, B., 219, 220
Peterson, G. M., 183
Peterson, L. O. T., 118, 127

Peterson, W. H., 381
Pettey, F. M., 112, 252, 253, 264
Peyerimhoff, P. de, 106
Pfeiffer, H. H., 19, 20
Pfrimmer, T. R., 379
Philip, C. B., 399
Philips, G., 292
Phillips, C. M., 280
Phillips, J., 259
Pickles, A., 236
Peipho, H., 216
Pierce, W. D., 190
Pimentel, D., 324
Pingale, S. V., 280, 285, 287
Piquett, P. G., 333
Planet, L., 129
Platt, R. B., 186
Plavilstshikov, N. N., 116, 117
Plotz, H., 400
Plummer, C. C., 194
Poisson, R., 1, 5, 13
Polanyi, M., 357
Polen, P. B., 336
Polivka, J. B., 279, 285, 290
Pollard, D. G., 111
Pollitzer, R., 397, 409
Pomerat, C. M., 17, 32, 34
Popov, I. D., 381
Porter, B. A., 344
Porter, C. E., 101
Porter, J. E., 420
Postiglione, M., 422, 423, 424, 426
Potter, C., 313, 321, 371, 374
Potts, S. F., 43
Powsner, L., 185
Poyarkoff, E., 14
Pradhan, S., 185, 321
Pratt, H. D., 402
Prebble, M. L., 192
Precht, H., 218
Predtetchenskii, S. A., 189, 191
Prescott, J. A., 189
Preston, A. P., 350, 354
Price, W. A., 324
Price, W. H., 34
Prince, F. M., 398, 399
Pringle, G., 423, 424, 426, 427
Pringle, W. L., 260, 266
Pritchard, A. E., 354
Proverbs, M. D., 321
Pruthi, H. S., 110
Pryor, M. R., 266, 269
Przibram, H., 60, 68
Psota, F. J., 126
Puck, T. T., 21, 25
Purrmann, R., 65, 66
Pyenson, L., 352

Q

Quarterman, K. D., 327
Quayle, H. J., 183, 198
Quayle, J. R., 63
Quilis Perez, M., 190
Qutub-ud-Din, M., 423, 428

R

Rabb, R. L., 280, 292, 294
Rachou, R. G., 422, 423, 425
Radha, K., 281, 287, 291
Rafaila, C., 381
Raffensperger, E. M., 372
Ragab, H. A., 34
Rahim, A., 423, 424, 425
Rainwater, C. F., 378
Ramachandran, S., 110
Ramakrishna, V., 422, 427
Ramsay, R. W., 26
Ramsey, L. L., 331, 338
Rao, H. H., 111
Rao, V. V., see Venkat Rao, V.
Raschig, H., 52
Rasmuson, B., 311
Ratcliffe, F. N., 403
Rateau, J., 423, 424, 426
Raw, F., 371
Rawlins, W. A., 374, 380
Raybaud, A., 394
Reali, G., 355
Redder, A. M., 335
Reed, A. C., 407
Reed, J. R., 379
Regnier, R., 116
Rehman, K. A., 110
Rehn, J. A. G., 89
Reid, E. T., 428
Reid, J., 109
Reid, J. A., 326
Reigel, A., 259
Reineck, G., 128
Reiner, C. E., 278, 289, 295
Rembold, H., 52
Remmert, L. F., 304
Renner, M., 46, 210, 215, 225
Reymond, A., 101
Reynolds, H. T., 363, 366, 369, 378, 379, 380
Rhein, W. v., 51, 53
Rhodes, A., 381
Ribbands, C. R., 210
Ribbands, L. R., 46
Richards, A. G., 144, 184, 185
Richards, O. W., 78, 162
Richet, P., 422, 424, 429
Riddick, J. A., 336
Ridpath, M. G., 381
Riedel, F. A., 10
Riker, A. J., 381
Rings, R. W., 350
Ripper, W., 104
Ripper, W. E., 343, 357, 363, 378
Ris, H., 20, 33
Risbec, J., 110
Ris Lambers, D. H., see Hille Ris Lambers, D.
Ristich, S. S., 373
Rivers, C. F., 278, 279
Rivola, E., 423, 424, 426
Rizki, M. T. M., 5, 7
Roan, C. C., 186
Robertson, C., 123
Robertson, R. L., 379
Robertson, W. R. B., 18, 25, 33
Robins, E. L., 313
Robinson, T. S., 63
Rockwood, L. P., 281, 285, 287, 291
Roeder, K. D., 303, 305, 306, 311, 312
Roegner-Aust, S., 284, 295
Roemhild, G., 266
Rohwer, S. A., 128
Rooseboom, M., 2, 9
Rösch, G. A., 46
Rosický, B., 293, 392
Ross, H. H., 90, 357
Ross, J. D., 34
Ross, R., 416
Rota, P., 355
Roth, L. M., 44, 45
Roth, W., 330, 338
Rothenbuhler, W. C., 175, 176
Rothschild, M., 392, 407, 408
Rothschild, N. C., 391, 392
Roussel, J. S., 379
Rovelli, G., 406
Roy, B. B., 422, 427
Rozeboom, L. E., 93, 94
Rubtzov, I. A., 184, 185, 189, 190, 192, 196
Rudd, R. L., 381
Ruggles, A. G., 102
Ruiter, L. de, 208
Ruiz Castro, A., 127
Rumreich, A., 400, 401
Rumreich, A. S., 401
Rungs, C., 117
Rupert, L. R., 80
Russell, P. F., 415-34; 415, 417, 418, 419, 420, 422, 423, 424, 425, 426, 427, 428, 429
Rutland, J. D., 313
Ryan, F. J., 185

S

Saalas, U., 128
Sabatino, F. J., 337, 338
Sacharov, N. L., 187
Sacklin, J. A., 304
Sailer, R. I., 77

St. Amand, G. S., 32
Sakagami, S. F., 223
Salkeld, E. H., 313, 374
Salt, G., 8, 166, 167, 169, 171
Salternik, Z., 423, 424, 427
Sanborn, C. E., 128
Sanders, D. F., 425
Sanderson, E. D., 186, 190, 191, 192, 193
Sandias, A., 49
Sanford, K. K., 22, 25, 32
Sang, J. H., 24, 27
Sankey, J. H. P., 188
Saraiva, A. C., 111
Sassuchin, D., 405, 408, 409
Satoh, K., 42
Sautet, J., 422, 426
Savage, A. A., 1
Savary, A., 345
Savely, H. E., 102, 105, 128, 130
Savit, J., 306, 307, 310, 311
Scales, A. L., 379
Schaerffenberg, B., 281, 283
Scheardt, H. H., 324
Schechter, M. S., 320
Schenk, A., 280, 287, 290
Scherer, W. F., 17, 32
Schiedt, U., 70, 71, 72
Schiffer, M., 236
Schilling, E. L., 31
Schiödte, J. C., 116
Schlehuber, A. M., 142, 146
Schmeider, R. G., 175
Schmid, H., 52
Schmidt, E. L., 20, 21, 22, 23, 27, 32, 33
Schmidt, K. P., 77, 81, 162, 183, 184
Schmidt, L., 292
Schmidt, R. S., 210, 227
Schmidtmann, M., 18
Schnathorst, W. C., 368
Schneider, D., 41, 42
Schneider, F., 7, 165, 166
Schneider, G. W., 354
Schneiderman, H. A., 21, 22, 24, 25, 27, 28, 30, 32, 33, 164
Schneirla, T. C., 210, 215, 216, 223
Schoeller, M., 66
Schomann, H., 103
Schoof, H. F., 324
Schroeder, W. T., 366, 367, 373
Schuch, K., 104
Schultz, E. F., Jr., 354
Schultz, F. N., 62
Schvester, D., 291
Schwardt, H. H., 324, 373
Schwartzman, G., 338
Schwarz, E. A., 115
Schwedtfeger, F., 329
Schwinck, I., 42, 43, 67, 217, 220
Schwitzgebel, R. B., 111
Scott, H. E., 374
Scudder, H. I., 324
Seamans, H. L., 199
Sellers, W. F., 264
Sellke, K., 201
Sengün, A., 42
Senior-White, R., 422, 427
Setty, H. T. R., 111
Sewell, T. G., 259, 268
Shafik, M., 110
Shands, W. A., 281
Shankland, D. L., 310
Shaposhnikov, G. Ch., 153
Sharif, M., 392
Sharma, H. N., 110
Sharma, M. I. D., 427
Sharp, D., 119
Shatoury, H. H. el, 1, 5, 14
Shaw, E. I., 21, 23, 26, 27, 29, 33
Shaw, J. C., 333
Shedley, D. G., 351
Shelford, V. E., 102, 124, 125, 126, 183, 184, 186, 190, 192
Shelmire, B., 401
Shepard, R. F., 188
Sheppard, N., 63
Shibata, K., 187, 199
Shibuya, T., 201
Shiraki, T., 109, 110, 111, 112
Shu-Chen, C., 111
Shulov, A., 200
Shute, P. G., 422
Shvetsova, O. I., 281, 292
Siegler, E. H., 327, 346, 350
Sikes, E. K., 408
Silveira, M. M. da, 326
Silverman, P., 336
Simič, Č., 429
Simmonds, F. J., 163, 164, 169, 259
Simmonds, H. W., 259, 262, 267, 272
Simmons, S. W., 326, 409
Simond, P. L., 394
Simpson, G. G., 80
Simpson, G. W., 281
Singh, B. N., 110
Singh, J., 422, 423, 424, 425, 426, 427, 428, 429
Sivik, F. P., 324
Skoblo, I. S., 165
Smallman, B. N., 306, 311, 312, 333
Smaragdova, N. P., 242
Smirnov, B. A., 281, 291
Smirnov, D. A., 116
Smith, A., 423, 429
Smith, A. D., 190
Smith, B. D., 144, 151
Smith, C. E., 373
Smith, E. J., 313, 344, 345, 350, 351
Smith, F. E., 248
Smith, F. F., 333
Smith, G. S., see Stace-Smith, G.
Smith, H. J., 313
Smith, H. P., 253, 259
Smith, H. S., 166, 183, 236, 237, 245, 251
Smith, J. M., 167, 170, 266
Smith, K. M., 278, 279
Smith, L. C., 347, 348
Smith, L. M., 147
Smith, O. J., 169, 178, 284
Smith, R. A., 175
Smith, R. E., 183
Smith, R. F., 188, 327
Snapp, O., 351
Snodgrass, R. E., 392
Soenen, A., 350
Sokolov, A. M., 145
Sokolova, R. A., 145
Solomon, M. E., 193, 235, 236, 251, 356, 357
Sorby, H. C., 62
Sörensen, N. A., 61
Soulage, J., 422, 424, 429
Speicher, B. R., 173
Speicher, K. G., 173
Spencer, E. Y., 303, 311
Spencer, G. J., 128
Speyer, E. R., 239
Spieth, H. T., 229
Spurway, H., 225
Srisukh, S., 60, 61, 68, 69
Srivastava, B. K., 366, 371, 378
Stace-Smith, G., 117
Stage, H. H., 102
Stahl, W. H., 45
Stanley, W. W., 379
Stansbury, R. E., 331
Starks, K. J., 372, 373
Starnes, O., 372
Staub, A., 349
Staudenmayer, T., 313
Stavraky, G. W., 308
Stay, B., 45
Stebbing, E. P., 108, 118
Stebbins, G. L., Jr., 77, 81
Steer, W., 356
Steiner, L. E., 259
Steiner, L. F., 346, 353
Steinhart, W., 424, 425
Steinhaus, E. A., 103, 277, 278, 279, 280, 282, 283, 284, 285, 287, 288, 289, 292, 293, 294, 295, 408
Steinhausen, W., 349
Stellwaag, F., 185
Stelzer, L. R., 351
Stenseth, C., 355
Stephens, J. M., 280, 284, 287, 288, 290
Stephens, R. M., 346
Stern, C., 20, 25, 33
Sternburg, J., 305, 307, 310

Steudel, W., 367, 380
Stewart, M. A., 406
Stokes, B., 93
Stoll, N. R., 288
Stone, M. W., 363, 364, 371
Stone, W. S., 82, 83, 93
Storrs, E. E., 332, 334, 335, 336
Stowe, B. B., 380
Stoy, R. H., 240
Strickland, W. B., 324
Stride, G. O., 220
Strong, F. E., 369, 372, 373
Stroyan, H. L. G., 139-60
Stumper, R., 48
Sturm, H., 209
Subbarow, Y., 26
Sudd, J. H., 211
Sullivan, C. R., 187
Sullivan, W. N., 324, 328, 331
Sulzdalskaya, M. V., 281
Sumerford, W. T., 326
Summerland, S. A., 346, 347, 348
Summers, W. A., 405
Sun, J. Y. T., 321, 333, 334
Sun, Y. P., 319, 321, 333, 334
Suomalainen, E., 87
Sussman, A. S., 286, 287, 292
Suzuki, T., 326
Swart-Füchtbauer, H., 292
Sweet, W. C., 426
Sweetman, H. L., 184, 185, 195, 197, 252, 259, 260, 269, 270, 271
Swellengrebel, N. H., 409
Swenson, K. G., 366, 367
Swezey, O. H., 102, 108, 111, 254, 255, 258, 260, 261
Swift, J. E., 327, 378
Swirski, E., 139, 142, 145, 147, 151, 156
Sy, M., 348
Syverton, J. T., 34
Szirmai, J., 284, 286, 287, 292

T

Taira, T., 68
Takakusa, S., 18
Takeda, K., 19
Takei, S., 337
Talbot, M., 185
Taliaferro, W. H., 403
Talibili, S. A., 423, 428
Tam, N.-D., 45
Tambs-Lyche, H., 146
Tamura, S., 23, 33
Tanada, Y., 277-302; 279, 280, 283, 285, 286, 287, 288, 289, 292, 294
Tanner, C. C., 371
Tarr, S. A. J., 378
Tarrter, A., 68
Tashiro, H., 279, 290, 291
Tattersfield, F., 321
Tauber, O. E., 3, 7
Taylor, A., 19, 33
Taylor, G. G., 346, 349
Taylor, H. S., 110
Taylor, L. R., 148, 149
Tehon, L. R., 189, 191
Telford, H. S., 366, 371, 372
Teotia, T. P. S., 324
Terriere, L. C., 304, 332
Teterovskaya, T. O., 185
Tew, R. P., 352, 354
Théodoridès, J., 292
Thistle, A., 268, 269, 270, 272
Thomas, C. A. 371
Thomas, F. J. D., 371
Thompson, C. G., 278, 279, 281, 285, 289, 290, 293, 294, 295
Thompson, J. A., 394
Thompson, J. L. C., see Cloudsley-Thompson, J. L.
Thompson, W. L., 281
Thompson, W. R., 235, 236, 251, 260
Thomsen, M., 192
Thomson, D. L., 65
Thomson, H. M., 283, 284, 286
Thomson, R. C. M., see Muirhead-Thomson, R. C.
Thomson, R. H., 62
Thornthwaite, C. W., 186, 189
Thorpe, W. H., 80, 81, 83, 106, 167, 179
Tiêu, N.-C.-, see Nguyên-Cong-Tiêu
Tiflow, W., 405, 408, 409
Tiggelovend, L. M. J., 111, 113
Tighe, J. F., 331, 338
Tillyard, R. J., 118, 122, 124, 125, 252, 254, 269
Timon-David, J., 59
Tinbergen, N., 207, 209, 212, 219, 224
Tippmann, F. F., 118, 121, 123, 128
Tipton, S. R., 32
Tischer, J., 60
Tisdale, E. W., 259, 266
Tixier, R., 69
Tobias, J. M., 25, 306, 307, 310, 311
Todd, A. R., 62, 63, 64
Tolley, H. R., 183
Tomizawa, C., 304, 315
Toole, E. H., 364
Törnblom, O., 220
Toumanoff, C., 7, 280, 283, 285, 286, 287, 289, 290, 293
Townes, M., 128
Townsend, G. F., 52, 344
Toyama, T., 326
Tracey, K. M., 26
Trägardh, I., 120
Trager, W., 19, 21, 23, 25, 26, 27, 28, 32, 34
Traub, A., 304
Traub, R., 392
Travis, B. V., 325, 326
Trebouse, J., 330, 338
Treherne, J. E., 143
Trewartha, G. T., 189
Trofimov, G. K., 423, 424, 429
Tsao, C. H., 324, 328
Tschesche, R., 66
Tseng, P., 424, 426
Tsuneki, K., 213, 216, 228
Tuppy, H., 69
Turner, N., 324, 373
Turzan, C. L., 344
Tusa, C., 381
Twarog, B. M., 311, 312

U

ud-Din, M. Q.-, see Qutub-ud-Din, M.
Ullyett, G. C., 168, 236, 237, 240, 290
Underhill, J. C., 322
Unger, K., 150
Ureña Hernandez, L., 422, 426
Uriarte, L., 392
Usinger, R. L., 77, 84, 88, 95
Utida, S., 235, 243
Uvarov, B. P., 99, 183, 184, 190, 194, 195

V

Vago, C., 27, 29, 30, 31, 278, 279, 280, 284, 287, 289, 290, 291, 292
Valcarce, A. C., 378, 379, 380
Valentine, I. M., 44
van Asperen, K., see Asperen, K. van
VanBaalen, C., 52
Van der Goot, P., 263
Van der Kloot, W. G., 215
van de Vrie, M., see Vrie, M. van de
Van Dinther, J. B. M., 373
van Emden, F. I., see Em-

den, F. I. van
van Esch, I., see Esch, I. van
van Hoof, H. A., see Hoof, H. A. van
Van Iersel, J. J. A., 224
Vănková, J., 289
Van Soest, W., 140
Vanwetswinkel, G., 350
Varela, G., 399
Vargas, L., 422, 424, 427
Varley, G. C., 166, 235, 241, 245
Varossieau, W. W., 209, 219
Vasiljević, L. A., 280, 286
Vayssière, P., 110
Veber, J., 282, 289, 294
Venegas, J. A. C. y, see Concha y Venegas, J. A.
Venkataraman, K., 62
Venkat Rao, V., 422, 423, 424, 426, 427
Villiers, A., 101, 110, 112
Viscontini, M., 52, 66, 67
Vivino, A. E., 52
Vleugel, D. A., 208
Vogel, G., 223
von Butovitsch, H., see Butovitsch, H. von
von Frisch, K., see Frisch, K. von
Von Hörmann-Heck, S., 209, 228
von Linden, M., see Linden, M. von
Voogd, S., 47, 48
Voûte, A. D., 235, 236
Vowles, D. M., 210, 211
Vrie, M. van de, 352
Vuillaume, M., 210

W

Waddington, C. H., 1, 14
Wagenknecht, A. C., 313
Wagner, J., 392
Wagner, R. P., 32
Wain, R. L., 381
Waite, H., 416
Walker, R. L., 379
Wallace, G. E., 167
Waller, E. F., 398
Walkley, L. M., 128
Wallis, R. C., 284, 292, 295
Walsh, B. D., 79, 106
Walthers, G. E., 372
Wangersky, P. J., 235, 246
Ward, C. Y., 367
Ward, J., 325, 328
Wardle, R. A., 162, 406
Wason, E. J., 346
Wasserburger, H. J., 338
Watanabe, S., 245
Waterhouse, D. F., 10, 143, 144
Waterston, J. M., 110
Watkins, J. C., 63, 64
Watson, E. A., 404
Watson, M. A., 142
Wattal, B. L., 422, 423, 427
Wave, H. E., 281
Way, M. J., 144, 145
Waymouth, C., 26, 27, 32
Weatherley, A., 312, 313, 314
Weatherley, P. E., 141
Weaver, J. E., 201
Weaver, N., 51, 208, 224
Weber, P. W., 112, 272
Webley, D. P., 3, 374
Webster, F. M., 117
Wedemeyer, J., 185
Weeks, W. F., 367
Weiant, E. A., 305, 306
Weidmann, U., 226
Weih, A. S., 209, 221, 223
Weiser, J., 278, 282, 283, 284, 285, 288, 289, 291, 292, 293, 294
Weis-Fogh, T., 218
Weitz, B., 418
Welch, H. E., 278
Wellington, W. G., 148, 183, 184, 186, 187, 188
Welsh, J. H., 306
Wenyon, C. M., 404
Werme., E. M., 19
Wertheim, G., 151
Wertman, K., 400
West, A. S., Jr., 193
West, L. S., 415, 417, 418, 419, 422
Westlake, W. E., 328
Weyer, F., 34
Whalen, M. M., 286
Wharton, D. R. A., 44, 45
Wharton, M. L., 44, 45
Wheeler, C. M., 407
Whitaker, W. D., 378
Whitcomb, W. D., 351
White, M. J. D., 83, 87, 89, 93, 173, 174, 175
White, P. R., 17, 33
White, R. S., see Senior-White, R.
White, R. T., 277, 279, 290, 291
Whiting, P. W., 172, 173, 174, 175
Whittaker, V. P., 312
Wiedbrauck, J., 215, 216
Wiedner, H., 116
Wieland, H., 68
Wiesmann, R., 346
Wigglesworth, V. B., 1-16; 1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 69, 73, 144, 146, 303
Wikén, T., 279, 287, 294
Wilbur, D. A., 111
Wilcoxon, F., 330, 331
Wildbolz, T., 279, 294, 349
Wilkes, A., 185, 239
Willard, A., 424, 428
Wille, H., 279, 287, 294
Williams, C. B., 184
Williams, C. M., 20, 21, 22, 23, 27, 30, 32, 33, 215
Williams, D., 111
Williams, F. X., 271
Williams, J. R., 251, 253, 254, 256, 258, 267, 268
Williams-Ashman, H. G., 303
Williamson, M. H., 235
Willis, E. R., 44
Willmer, E. N., 17
Wills, J. H., 313
Wilson, C. E., 325
Wilson, E. O., 85, 86
Wilson, F., 251, 253, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 266, 268, 269, 271, 272
Wilson, H. G., 326
Wilson, I. B., 312, 313
Wilson, K. M., 338
Wilson, L., 219
Wingo, C. W., 325
Winteringham, F. P. W., 303-18; 303, 304, 306, 311, 312, 313, 314, 315, 316
Winton, M., 379
Witherspoon, J. P., 186
Witman, E. D., 345
Witt, A. A., 242
Witt, J. M., 330, 331
Witt, P. N., 214
Wittekindt, W., 226
Wolcott, G. N., 111, 291
Wolf, J. P., III, 379
Wood, F. D., 404
Wood, H. C. S., 68
Woodruff, N., 324
Woods, A., 374
Woods, R. W., 428
Woodward, R. C., 371
Woodward, T. E., 402
Woodworth, C. E., 371
Workman, W. G., 400
Wright, D. W., 378
Wright, F. N., 426
Wright, J. M., 368
Wright, W. H., 405
Wu, Lien-Teh, 409
Wu, Y. T., 424, 426
Wyatt, G. R., 24, 26
Wyatt, S. S., 3, 21, 22, 24, 25, 26, 27, 28, 29, 30, 31, 32
Wylie, W. D., 329

Y

Yamaguchi, K., 23, 33

Yamaki, T., 380
Yamasaki, S., 404
Yamasaki, T., 305, 309
Yeager, J. F., 4, 11, 12
Yeomans, A. H., 328
Yersin, A., 394
Young, H. C., Jr., 350
Yushima, T., 311, 313
y Venegas, J. A. C., see Concha y Venegas, J. A.

Z

Zachau, H. G., 41
Zaheer, S. H., 315
Zappetini, G., 259
Zeller, H., 12
Zentmyer, G. A., 381
Ziady, S. E., see El Ziady, S.
Ziegler-Günder, J., 65, 67, 68
Zimmack, H. L., 282, 283, 286
Zimmerman, E. C., 108
Zinsser, H., 400, 401
Zippelius, H. M., 209
Zitcer, E. M., 32
Zoch, E., 52
Zoebelein, G., 178
Zulueta, J. de, 423, 426, 428
Zurakowski, W., 367
Zweig, G., 95
Zweigelt, F., 142, 154

SUBJECT INDEX

A

Acaena sanguisorbae, 259, 270-71
Acaenitini, 128
Acanthocinus, 105, 115, 119
Acanthocinus aedilis, 129
Acanthocinus nodosus, 130
Acanthoderini, 122
Acanthomigdolus, 127
Acanthophorus, 121
Acanthoscelides obtectus, 329
Acanthosciades, 122
Acanthospermum hispidum, 272
Acaricides, 354-55
Aceria tulipae (wheat curl mite), 366, 380
Acetylcholine, 311-12
ACH
  nerve metabolism in, 306, 307, 310
  in Periplaneta americana, 311
Acheta, 89, 227
Acmaeops, 127
Acmocera conjux, 128
Acomys witherbyi, 391
Acotyledon agilis, 242
Acrididae, 143
Acrocinus longimanus, 121
Acrostalagmus aphidium, 281
Actinociphalus parvus, 409
Acyrthosiphon pisum, 141, 143
Acyrthosiphon spartii, 144, 151
Adaptation, 121-27
Adelges piceae, 144
Adelges strobi, 62, 63
Adelgidae, 142
  heteroecy, 154, 155
  sex forms in, 146
Adelina tribolii, 248
Adipocytes, 1
Adisura atkinsoni, 280, 285
Adoxophytes orana, 349
Aedes, 420
Aedes aegypti
  bioassay of insecticides, 331-33
  as carrier of yellow fever, 391
  DDT effect on, 304
  effect of insecticides on, 304, 325, 327, 334
  haemocytes of, 2
  photomigration assay method of, 334-37
  tissue culture of, 24, 28
  vitamin deficiency, 53
Aeolesthis holosericea, 108
Aerenicopsis championi, 112, 261
Aeschna, 10
African migratory locust, see Locusta migratoria migratoriodes
Agapanthia, 127
Agapanthia dahli, 111
Agapanthia villosoviridescens, 117
Agapanthida, 122
Agathis festiva, 329
Agonoderus comma, 378
Agonoderus lecontei (seed-corn beetle), 378
Agrilus anxius, 78
Agrilus hyperici, 265
Agriotes, 371
Agriotes mancus, 372
Agriotes obscurus, 281
Agriotes sputator, 281
Agrippina bona, 409
Agromyza lantanae, 261, 262
Agrotis orthogonia, 198
Agrotis ypsilon, 379
Alaus, 128
Aldrin
  control by
    of carrot rust fly, 374
    of European apple sawfly, 352
    of flea beetles, 378
    of frit fly, 374
    of onion maggot, 374
    of plum curculio, 351
    of wireworms, 369-72
  fish sensitivity to, 337-38
  measurement of potency, 322
  measurement of action, 307-8
  neurophysiological disturbances, 308-9
  plant residual toxicity testing for, 331, 332, 333, 334
  seed treatment, 366, 378
  tests for residue of, 324
  T_{50} test and, 336
Aleyrodidae, 140
Alfalfa caterpillar, see Colias philodice eurytheme
Alfalfa snout beetle, see Brachyrhinus ligustici
Alfalfa weevil, see Hypera postica
Algae, 282-83
Allethrin, 322, 325, 336
Almond moth, see Ephestia cautella
Alnus, 92
Alysia manducator, 167
Amblycorypha, 89
American cockroach, see Periplaneta americana
Ametastegia glabrata, 353
Amino acids, 26-27
Ammophila, 212
Ammophila campestrist
  classification of, 227
  feeding habits, 94
  fighting among, 208
  nest building, 213
Amoebocyte, 10, 11
Amphimallon majalis, 279, 291
Amphipsylla rossica, 398
Anacolini, 122
Anacolus, 125
Anagasta kühniella (Mediterranean flour moth)
  haemocyte count, 4
  parasite-host relation, 238, 243
  pigments of, 66, 67, 72
  Sporeine in control of, 280
Anarsia spartiella, 259
Anasa tristis, 69
Anastrepha ludens (Mexican fruit fly), 200
Anaxipha, 89
Anchastus cinereipennis, 368
Aneflomorpha, 102, 112
Anelaphus nanus, 128
Aneristus, 169
Angitia, 168
Anopheles
  death rate, 418
  dispersal by transportation, 420
  host for malaria, 417
  and malarial parasites, 389
  reproduction rate, 418
  taxonomy of, 93
  vector density, 418
  as vectors of malaria species list, 420-31
Anopheles albimanus, 326
Anopheles culicifacies, 419
Anopheles fluviatilis, 417
Anopheles gambiae, 326, 419
Anopheles litoralis, 417
Anopheles maculipennis

atroparvus, 219, 224
Anopheles minimus flavirostris, 417
Anopheles quadrimaculatus
and dog heart worms, 405
haemocytes of, 2
insecticide residual toxicity, 325, 326
means of testing insecticidal residues, 333
Anopheles subpictus indefinitus, 420
Anopheles vagus, 326
Anoplius, 227
Anoplodera, 112
Anoplodera instabilis, 103
Anoplodera nitens, 110
Anoplodermini, 122, 127
Anoplophora macularia, 110
Anoplura, 390
Antheraea polyphemus, 30
Anthocyanins, 65
Antholcus varinervis, 259, 271
Anthonomus grandis (boll weevil), 379
Anthonomus pomorum (apple blossom weevil), 351
Anthoxanthins, 65
Antibiotics, 31, 292-93
Ants
and aphids, 155-56
communication in, 210-11
cyclic changes in behavior, 215-16
fat body of, 1
mimicry of, 124, 126
queen substance of, 48-49
see also specific names
Anurphis roseus, 353
Aonidiella, 94
Aonidiella aurantii (California red scale), 167, 170, 238
Apanteles glomeratus, 164, 294
Apanteles medicaginis, 167
Apanteles solitarius, 166
Apatophysis, 126
Aphelenchoides besseyi, 380
Aphelinidae, 169
Aphid
aphins of, 62-64
biology of, 139-60
control
by systemic materials, 379
damage to weeds, 260
evolution of host relations
climatic factors, 156-57
degree of adaptation, 154-55
dispersal hazards, 155
growth patterns, 155
myrmecophily, 155-56
nonparasitic phases
forms, 146-48
functions, 144-48
host finding, 150-52
host selection, 150-52
migration, 148-50
parasitic life
degeneration, 140
feeding, 140-43
field populations, 144-46
nutrition, 143-44
parthenogenesis in, 87
Aphididae
host relationship, 155
pigments of, 62-64, 65
Aphidoidea, 139
Aphis craccivora, 148
Aphis fabae, 63
feeding of, 140, 142
form determination, 147
host range, 153
host selection, 151, 152
migration of, 148, 149, 150
number of progeny, 140
nutrition of, 143
population control, 144, 145
Aphis farinosa, 146
Aphis gossypii (cotton aphid), 77-98, 379
Aphis pomi, 353
Aphrins, 62-64
Aphytis chrysomphali, 167
Aphytis lingnanensis, 167, 238
Aphytis maculicornis, 166
Aphytis mytilaspidis, 237-38
Apion antiquum, 270
Apion neofallax, 270
Apion ulicis, 269
Apis florae, 225
Apis mellifera (honey bee)
communication in, 210-11
dancing of, 225-26
DDT susceptibility, 327
flower feature attractions, 221
haemocytes
count, 5
nutritive function, 11
types of, 2
insecticide residue testing on, 323
marking scents, 45-46
mating of, 175-76
queen substance of, 47-48
royal jelly
chemistry of, 52-53
determining factor, 51-53
mechanism of action, 53
somatic diploidy, 173
Apoderus coryli, 207
Apomecyna, 111
Apomecyna binubila, 120
Apomycini, 124
Aporia crataegi, 294
Aptenopedes sphenarioides, 89
Apterocaulus, 126
Aradus cinnamomeus, 281, 290
Aramite, 328
Archips argyrospila, 353
Archips fumiferana, 95
Archips rosana, 164, 353
Argynnis, 222
Argynnis paphia, 43, 220
Argyrotaenia velutinana (red-banded leaf roller), 279, 347, 349
Arhopalus, 112, 119
Arilus cristatus, 128
Aromia moschata, 116
Arrhenotoky, 172
Arsenicals, 371
Arthrochlamys, see Chlamisus
Arvicanthis testicularis, 391
Ascogaster, 162
Aseminae
cryptic coloration, 122
distribution, 100, 102
host specificity, 105
mating, 117
oviposition of, 119
sound production, 118
symbiotes of, 103
Asilidae, 128
Ash-gray blister beetle, see Epicauta fabricii
Aspergillus flavus, 286
Aspergillus ripens, 281
Aspidaphium, 142
Aspidiotus forbesi, 353
Aspidiotus perniciosus (San Jose scale), 193, 353
Ataxia hubbardi, 111
Ataxia sulcata, 111
Athesapeuta cyperi, 271-72
Atimia, 100
emergence of, 112
host specificity, 106, 107
Atimia dorsalis, 112, 120
Atomaria linearis (pygmy mangold beetle), 378
Atriplex confertifolia, 260
Attacus, 68
Attagenus piceus, 323
Atta sexdans rubripilosa, 46
Attelabus nitens, 207
Auchenorrhyncha, 140
Aulaconotus pachypeziodes, 120, 122
Australian locust, see Chortoicetes terminifera
Austroicetes cruciata, 196
Austroicetes pusilla, 89
Austrotortrix postvittana, 347, 349
Automeris aurantiaca, 216

B

Bacillus cereus, 279, 286
  degrees of virulence, 287
  persistence of, 290
  viability of, 288
Bacillus cereus var. alesti, 279
Bacillus popilliae, 279, 291
Bacillus sotto, 279, 286, 293
Bacillus thuringiensis, 279, 283
  degrees of virulence, 287
  effect of antibiotics on, 293
  effect on Polistes exclamans, 294
  host susceptibility, 286
  persistence of, 290
  spread of, 285
  viability of, 288
Bacteria, 279-80
Bactra truculenta, 271-72
Bairamlia fuscipes, 408
Barathra brassicae (cabbage armyworm), 322
Baryssinus leguminicola, 111
Batocera lineolata, 129
Batocera rufomaculata, 113
Batocera wallacei, 121
Bayer 25141, 373
Bayer 25198, 376
Beauveria, 281
  degrees of virulence, 287
  viability of, 288
Beauveria bassiana
  Cerambycidae susceptibility, 129
  Lepidoptera resistant to, 286
  microbial control, 281, 283
  optimum germination, 291
Beauveria tenella, 281
Bed bug, see Cimex lectularius
Bee
  flower feature attractions, 221
  intruders, 209
  mimicry of, 125, 126
  see also specific names
Beet leafhopper, see Circulifer tenellus
Behavior of insects, see Ethological studies of insect behavior
Belostoma indica, see Lethocerus indicus
Bembix, 224
Bembix brullei, 228
Bembix nipponica, 213, 216
BHC (benzene hexachloride), 371
  control by
    of European apple sawfly, 352
    of flea beetles, 378
    seed-corn maggot, 373
    seed treatment, 378
    of wireworms, 371, 372
    of woolly apple aphid, 350
  malaria eradication, 421
  plant residual toxicity testing for, 332
  residual toxicity of, 323, 326, 327, 330
  residue testing for, 323
  soil residual toxicity of, 329
γ-BHC
  control by
    of carrot rust fly, 374
    of flea beetles, 374, 378
    of frit fly, 374
  lethal action of, 310
  measurement of potency, 322
  residue testing methods for, 321
  seed treatment, 378
  wireworm control, 371, 372
Bidi-bidi, 270-71
Bildungsherde, see Haemopoietic organs
Bile pigments, 68-69
Bioclimatics, 183-206
  analysis, 188-90
  biological aspects of, 184-88
  climatic indicators, 188-89
  climatic observation, 188
  correlations, 190-97
  definition of, 183
  humidity, 185-86
  light intensity, 187
  rain and winter cold, 186
  snow, 186
  temperature, 184-86
  thermal constant and distribution, 191-92
  use in forecasting distribution, 197-201
Biological control
  of cacti, 112
  host-parasite populations, 243-45
  parasitic Hymenoptera, 161-80
  of weeds with insects, 251-76
    current efforts, 272-73
    examples of, 260-72
    host specificity, 254-55
    principles and concepts, 253-54
    probability of success, 257-60
    risks of introduction, 254-55
    starvation tests, 255-56
    unfounded criticisms of, 256-57
Black sage, see Cordia macrostachya
Blapstinus, 379
Blatta orientalis, 7
Blattella germanica, 2, 44, 313
Blepharomastex acutangulalis, 261, 262
Blissus leucopterus (chinch bug), 327
Blood cell, 1-16
  organs forming, 2-3
  see also Haemocyte
Blowfly, 26
Bombus hortorum, 46
Bombyx mori (silkworm)
  epidermis survival, 24
  food ingestion rate, 141
  haemocyte count, 3
  hormone influence on behavior, 216
  microsporidia in, 283
  and Nosema bombycis, 34
  pebrine of, 282
  pheromones of, 39
  pigments in
    anthoxanthins, 65
    carotenoid, 60, 61
    cytochrome-C, 69
    ommochromes, 72-73
    pterins, 66, 67, 68
  response to female scent, 217
  sex attractant of, 40, 42
  survival in Wyatt's medium, 25
  tissue culture, 19, 20, 22, 23
    amino acids, 26
    energy source of, 26
    hanging drop in, 30
    hormones in, 28
    media for, 29
    nucleic acids in, 27
    tissue extracts, 28
Borrelia recurrentis, 389
Brachycaudus helichrysi, 146
Brachycera, 226
Brachyrhinus ligustici, 283, 293
Brachyrhinus oribricollis, 351
Brachysomida, 112
Brachysomida tumida, 103
Bracon, 175, 176
Bracon greeni lefroyi, 238
Bracon hebetor
  distribution of progeny, 240
  genetics of, 173
  host-parasite range, 238
  venom of, 180
Braconidae, 127
Bradynema strasseni, 128
Brevicoryne brassicae, 141, 148, 150
Brill's disease, 399, 400

Bronze birch borer, see Agrilus anxius
Bryobia arborea, 346, 354
Bucculatrix thurberiella, 379
Buprestidae, 123
Byturus bakeri, 78
Byturus rubi, 78

C

Cabbage armyworm, see Barathra brassicae
Cabbage looper, see Trichoplusia ni
Cabbage seed-stalk curculio, see Ceutorhynchus quadridens
Cabbage webworm, see Hellula undalis
Cactoblastis cactorum, 253, 263-65
Cactoblastis doddi, 264
Cactophagus spinolae, 264
Calamobius filum, 111
California oakworm, see Phryganidia californica
California red scale, see Aonidiella aurantii
Caliptamus, 227
Callaphididae
  host bond, 155
  host colonization, 153
  migration in, 148
  sex forms in, 146
Callichromini, 110, 116, 123
Callidiellum, 106
Callidiini, 102, 105, 117
Callidium, 105-7
Calligrapha, 92
Callimoxys, 100
Calliphora, 53
  blood coagulation, 9, 10
  haemocyte count, 5, 6
  haemopoietic organ of, 3
  metamorphosis in, 6
  tissue culture, 18
    media for, 29
Calliphora erythrocephala, 70, 304, 312
Callisto geminatella, 349, 353
Callitroga hominivorax, 78, 334
Callitroga macellaria, 334
Callosamia promethea (promethea moth), 21, 30
Callosobruchus chinensis, 243, 245
Callosobruchus maculatus, 243
Calomel seed treatment, 373, 374
Calopteryx, 208, 217, 223
Calopteryx splendens, 209, 221
Capitophorus, 153
Carassius auratus, 337
Carausius, 12
Carausius morosus
  haemocyte role, 8, 9
  pigments of, 60, 68
Carausius septempunctata, 60
Caroliniella aenescens, 110
Carotenoids, 60-61
Carpocapsa pomonella (codling moth)
  control of
    by bacteria, 280
    by DDT, 346, 347, 348
    by Diazinon, 348
    by Dilan, 347
    by EPN, 348
    by methoxychlor, 347
    by parathion, 347, 348
  delayed emergence, 345
  derivation of name, 344
  diapause induction, 344
  lead arsenate as stomach poison for, 345
  mite populations increase in, 346
  persistence of Bacillus cereus in, 290
  temperature requirements, 201
Carulaspis visci, 260
Casca, 169
Catabena esula, 261, 262
Cecropia moth, see Hylalophora cecropia
Cediopsylla, 403
Cediopsylla simplex, 398
Centaurea, 272
Centaurea solstitialis, 254
Cephalonomia waterstoni, 224
Cephalosporium, 281
Cephus cinctus (wheat sawfly), 199
Cephus pygmaeus, 167
Cerambycidae
  adaptations for subterranean existence, 126
  Batesian mimicry, 124-25
  body size, 121
  distribution of genera, 100
  ecology of, 99-138
  economic importance, 108-12
    field crops, 110-11
    flowers, 111-12
    forest products, 109
    forests, 108-9
    fruit trees, 110
    nut trees, 110
    seed-infesting, 111
    shade trees, 109-10
    vegetables, 110-11
  effect of humidity, 130
  ethological adaptation, 112-20
  feeding habits classified, 115-16
  form, 121
  geographical ranges of, 101, 102
  hibernation of, 120
  host specificity, 105-8, 117, 118
  larval food habits, 102-3
  mating, 116-17
  Mullerian mimicry, 125-26
  natural mortality, 127-30
  olfactory sense, 118
  oviposition of, 119-20
  physiological adaptation, 112-20
  sexual dimorphism, 121-22
  sound production, 118-19
  structural adaptations, 121-27
  symbiosis, 103-5
  temperature and, 129
  wood digestion, 103-5
Cerambycinae
  Batesian mimicry, 125
  bright coloration, 123
  cryptic coloration, 122
  distribution of, 100, 102
  feeding habits, 115
  host specificity, 105
  longevity of, 113-14
  mating, 117
  oviposition of, 119
  sexual pubescence, 122
  sound production, 118
  symbiotes of, 103
Cerambyx, 104
Cerambyx cerdo, 121, 129
Ceratitis capitata
  climatic influences on distribution, 191
  comparison of climates for, 198
  forecasting of distribution, 199
  limiting climatic conditions, 187
  Opius tryoni as control for, 252
Ceratophyllus acutus, see Diamanus montanus
Ceratophyllus columbae, 408
Ceratophyllus gallinae, 408
Ceratophyllus hirudinis, 404
Ceresium sinicum, 129
Ceroctenus, 122
Cestode infestations, 405-6
Ceutophilus, 82
Ceutorhynchus quadridens (cabbage seed-stalk curculio), 378

Chaetocnema, 374, 378
Chaetonodexodes marshalli, 259
Chagas's disease, 404
Chaitophoridae, 155
Chaitophorinella, see Periphyllus
Chalcidordea, 177
Chariessa, 127
Chauliognathus, 125
Chelinidea tabulata, 263, 265
Chelinidea vittiger, 259, 263, 265
Chelonus, 162
Chelonus annulipes, 165
Chelonus texanus, 169, 240
Chelosterna scabrator, 129
Chestnut blight, 110
Cheyletus eruditis, 242
Chinch bug, see Blissus leucopterus
Chionaspis furfura, 353
Chironomus, 2, 93
Chironomus plumosus, 3
Chlamisus, 81, 92
Chlamys, see Chlamisus
Chlordane
  control by
    of European apple sawfly, 352
    of plum curculio, 351
    of seed-corn maggot, 373
  fish sensitivity to, 337-38
  residue testing methods for, 322, 323, 324, 326, 327, 329, 332
  seed treatment, 378
  T_{50} test and, 336
α-Chlordane, 307
Chloridolum, 117
Chlorinated camphene, 326
Choristoneura fumiferana (spruce budworm)
  host mortality, 236
  microsporidia
    egg transmission, 283, 284
    infections, 286, 287
  taxonomy of, 79
Choristoneura pinus, 78
Chorthippus, 223
Chorthippus biguttulus, 209, 221
Chorthippus brunneus, 209, 221
Chortoicetes terminifera (Australian locust), 196
Chrysobothris, 130
Chrysolina gemellata, see Chrysolina quadrigemina
Chrysolina hyperici, 266, 267
Chrysolina quadrigemina, 258, 265-67
Chrysolina varians, 266
Chrysomela, 82, 93
Chrysomela hyperici, see Chrysolina hyperici
Chrysomela interrupta, 90
Chrysomela quadrigemina, see Chrysolina quadrigemina
Chrysomelidae, 14, 90
Cimex, 7
Cimex lectularius (bed bug), 322, 323
Cionus oleus, 65
Circulifer tenellus (beet leafhopper), 379
Citellus, 402
Citellus beecheyi, 397
Citriphaga mixta, 110
Classification, 118, 119, 139
  see also Taxonomy
Clidemia hirta, 259, 267
Climate
  analysis of, 189-90
  classification, 189
  climatographs, 190
  cold as limiting distribution, 192-94
  distribution, 189
  distribution of Cerambycidae, 99, 100, 101
  effect on aphids, 156-57
  heat as limiting distribution, 194-95
  indicators, 188-89
  moisture as limiting distribution, 195-97
  observations, 188
  see also Bioclimatics
Clivina impressifrons, 378
Closterus, 121
Clytanthus herbsti, 117
Clytini, 117, 123, 124
Clytoleptus, 100
Coagulation, blood, 9-10
Coccidia, 61, 282
Coccinella septempunctata, 60, 196
Coccinellidae, 60
Coccoidea, 140
Coccophagus, 169
Cockroach, 23, 26
  see also specific names
Cocoon construction, 215
Codling moth, see Carpocapsa pomonella
Coenonympha, 95
Coleomethia evaniiformis, 125
Coleoptera
  bacterial control of, 280
  carotenoids of, 60
  Cerambycidae
    ecology of, 99-138
  haemocyte count, 5
  Müllerian mimicry in, 125-26
  sex attractants, 43-44
  taxonomy, 90-93
  turning behavior, 227
Colias, 78
Colias boothii, 82
Colias eurytheme, 82 167
Colias hecla, 82
Colias lesbia, 280
Colias nastes, 82
Colias philodice, 82
Colias philodice eurytheme
  bacterial control, 280
  biological and microbial control, 293, 294
  virus control, 279, 290
Collembola, 209
Collyria calcitrator, 167
Colorado potato beetle, see Leptinotarsa decemlineata
Coloration
  behavior studies and, 208
  bright, 123-24
  contrasting, 123-24
  cryptic, 122-23
  and generalized escape behavior, 228
  mimicry
    Batesian, 124-25
    Müllerian, 125-26
  preference, 150, 220
  see also Pigments
Comperiella bifasciata, 94, 171
Compositae, 123
Compsosoma mutillarium, 125
Conoderus, 372
Conotrachelus nenuphar (plum curculio), 345, 350-51
Copidosoma, 179
Copidosoma koehleri, 177
Copulation, 209
Cordia macrostachya, 256, 259, 267-68
Corethra, 19, 20
Corixa punctata, 2
Corn earworm (see Heliothis zea)
Cortodera, 100, 112
Cortodera berkelyensis, 103
Corylobium, 153
Corythalia, 227
Corythalia xanthopa, 222
Costelytra zealandica, 285
Cotton aphid, see Aphis gossypii
Courtship
  behavior patterns in, 209
  song, 214
  types of in grasshoppers, 227
  see also Mating
Crabro cribarius, 44
Crambus bonifatellus, 282

Cremastobombycia lantanella, 261
Crithidia cleopatrae, 409
Crithidia ctenophthalmi, 409
Crithidia fasciculata, 53
Crocidosema lantanae, see Epinotia lantana
Crofton weed, 268
Crossidius, 103, 112, 123
Crustacea, 337-39
Cryptolaemus montrozieri, 252, 264, 265
Cryptomyzus, 153
Cryptomyzus ribis, 142, 143, 145
Cryptus inornatus, 163, 164, 240
Ctenicera aeripennis destructor, 78
Ctenicera pruinina noxia (Great Basin wireworm), 371, 372
Ctenocephalides canis
  anemia and, 406
  cestode infestations, 405
  filariasis transmission, 405
  salmonellosis transmission, 399
  typhus transmission, 401
Ctenocephalides felis
  cestode infestations, 405
  filariasis transmission, 405
  myxomatosis transmission, 403
  richettsial infections, 409
  typhus transmission, 401
Ctenophthalmus agyrtes, 398, 409
Ctenophthalmus assimilis, 398
Ctenophthalmus pollex, 398, 405
Ctenopsylla segnis, 398
Cucujus, 127
Culex, 93, 389, 420
Culex pipiens, 2
Culture
  of insect tissues, 17-38
Culture flasks
  types of, 30-31
Cyclic changes
  in behavior of ants, 215-16
Cyclocephala, 378
Cycloptilum, 89
Cylas formicarius, 201
Cyllene robiniae, 129
Cymatodera, 127
Cynthia, 28
Cyperus rotundus, 271-72
Cyphus myrmosarius, 125
Cyrtinus pygmaeus, 121
Cystospora, 109
Cytisus scoparius, 272
Cytokinesis, 20
  tissue culture, 20

D

Dactylopius ceylonicus, see Dactylopius indicus
Dactylopius coccus, 61
Dactylopius confusus, 259, 263, 265
Dactylopius indicus, 262, 263
Dactylopius newsteadi, see Dactylopius confusus
Dactylopius opuntiae, see Dactylopius tomentosus
Dactylopius tomentosus
  control of prickly pears, 252, 263, 264, 265
Dacus cucurbitae, 187, 199
Dacus dorsalis, 187, 199
Dahlbominus fuscipennis, 168, 239
Danaus chrysippus alcippus, 220
Daphnia, 6
Daphnia magna, 338
Daphnia pulex, 338
Darkling beetles, 379
Dasyneura, 93
Dasyneura mali, 353
DDA, 303
DDE, 303, 304
DDT
  comparison with aldrin, 309
  control of
    of apple blossom weevil, 351
    of apple flea weevil, 351
    codling moth, 346, 347, 348
    deciduous fruit insects, 343
    of fruit insects, 353
    murine typhus, 402
    of onion maggot, 373
    of plum curculio, 351
    of seed-corn maggot, 373
  effect on
    avian populations, 381
    oxidative metabolism, 303-5
  fish sensitivity to, 337-38
  and increase in Panonychus ulmi, 354
  lethal action of, 307
  malaria eradication, 421, 430, 431
  and microbial control, 293
  neuromuscular disturbances due to, 305-6
  residues of, 319
  residue testing methods
    cage method, 328-29
    coated cage method, 326-28
    coated plate method, 324-26
    impregnated cloth, 323
    impregnated paper, 321-22
    for plant, 330-37
    soil, 329
  T_{50} test and, 336
DDVP, 314
Deciduous fruit insects
  and their control, 343-62
    chemical, 355-57
Degeneration, 140
Demeton (Systox), 378-80
Dendrobias, 115
Dendrobias mandibularis, 118
Dendroctonus, 90
Dendroides bicolor, 130
Dermacentor variabilis, 322, 400
Derobrachus brunneus, 105
Derris, 319
Desmiphora, 122
Desmocerus, 103
Deuterotoky, 172
DFP, 312-14
Diabrotica undecimpunctata howardi (southern corn rootworm), 378
Dialges, 103
Diamanus montanus, 398
Diamond back moth, see Plutella maculipennis
Diapause, 163-66
Diastema tigris, 261, 262
Diataraxia oleraceae, 8
Diaxenes, 111, 113
Diazinon
  control by
    of seed-corn maggot, 373
    of woolly apple aphid, 350
  residue of, 325
  seed treatment, 367
Didinium nasutum, 242
Dieldrin
  control by
    of Brachyrhinus cribricollis, 351
    of European apple sawfly, 351, 352
    of fruit fly, 374
    of onion maggot, 373-74
    of seed-corn maggot, 373, 377
    of wheat bulb fly, 374
    of wireworms, 369-72
  effect on Periplaneta americana, 309, 310
  fish sensitivity to, 337
  malaria eradication, 421, 430
  measurement of potency, 322
  mechanism of action, 307
  plant residual toxicity testing for, 332, 334

residual toxicity tests, 324, 327
seed treatment, 366, 378
Dihammus cervinus, 113
Dilan
control by
of codling moth, 347
fish sensitivity to, 337-38
Dipetalonema reconditum, 405
Diplazon fissorius, 7, 8, 165
Diplazon laetatorius, 7
Diplazon pectoratorius, 166
Diplococcus pneumonia, 409
Dipodillus watersi, 391
Diprion, 192
Diprion hercyniae (European Spruce sawfly)
biological and microbial control, 293
temperature preference, 239, 292
virus infections, 278, 286, 287, 290
Diptera
cleaning behavior, 226
color preference, 224
embryonic determination, 23
haemocyte count, 4, 5
imaginal discs in, 33
as predators, 128
sex attractant, 116
taxonomy of, 93-94
tissue formation, 10
Dipus jaculus, 391
Dipylidium caninum, 405
Dipylidium sexcoronatum, 406
Dirofilaria immitis, 405
Di-syston, 373
control by
of cotton insects, 379-80
of onion maggot, 374
as systemic agent, 379-80
Dixippus morosus, see Carausius morosus
Doliops, 125
Dorcadion, 111, 115
Dorcasta cinerea, 111
Dorysthenes, 127
Drasterius dorsalis, 368
Drepanosiphum platanoides, 146, 148
Drosophila
cellular development, 1
cleaning behavior of, 226
courtship, 216, 217
growth factors of, 14
haemocytes of, 2
insecticide residue testing methods, 324
melanotic tumor of, 23
phagocytosis in, 7
sex determination in, 174
sexual isolation, 94
taxonomy of, 81, 93
tests for insecticide residue, 321
tissue culture, 20, 25
media for, 28, 29
vitamins, 27
tumors of, 33, 34
Drosophila aldrichi, 82
Drosophila melanogaster
γ-BHC residue testing with, 322
DDT residue testing with, 321
genetic studies on, 69
haemocyte count, 5
means of testing insecticidal residues, 333, 334
pigments of, 66, 68
soil residual toxicity of insecticides for, 329
Drosophila mulleri, 82
Drosophila tripunctata, 82
Drosophila viridis, 229
Drosophila willistoni, 4, 5
Dutch elm disease, 110
Dysaphis bonomii, 148
Dysaphis devecta, 146
Dytiseus, 18

E

Earias fabia, 238
Eastern field wireworm, see Limonius agonus
Eastern raspberry fruitworm, see Byturus rubi
Eburodacrys, 122
Echidnophora, 392, 403
Echidnophaga myrmecobii, 403
Echium plantagineum, 253
Ecology
Cerambycidae, 99-138
and mimics, 125
Ectohormones, see Pheromones
Elaphidionoides, 102
Elateropsis, 123
Eleodes, 378
Elymus caput-medusae, 257
Embryonic determination, 23
Emex australis, 270
Emex spinosa, 270
Empoasca maligna, 353
Empoasca solana, 379
Empusa, 285
Enaretta castelnaui, 111
Encarsia formosa
host-parasite population, 244
host-parasite relationship, 246
temperature effect on, 168, 239
Encyclops, 103
Encyrtus fuliginosus, 178
Endrin, 307, 369-72
Endrin, 307, 369-72
Enneaphyllus, 121
Entomophthora, 129, 281, 292
Entomophthora coronata, 292
Entomophthora exitialis, 292
Entomophthora virulenta, 291, 293
Ephestia
haemocyte
count, 3, 6
nutritive function of, 12
as host for parasite, 167
pigment of, 67
Ephestia cautella (almond moth), 193
Ephestia kühniella, see Anagasta kühniella
Epiblemum scenicum, 221
Epicauta fabricii, 78
Epidemiology
circumstantial, 416
of malaria, 415-34
mathematical, 416-20
Epilachna varivestis (Mexican bean beetle)
humidity and hibernation, 185
limits on distribution, 194, 197
seed treatment as control, 380
Epinotia lantana, 261, 262
Episomus chlorostigma, 125
Epistrophe balteata, 7, 165
Epistrophe bifasciata, 8, 165
Epitrix cucumeris (potato flea beetle), 380
Epitrix tuberis, 78
EPN
control by
of apple flea weevil, 351
of codling moth, 348
of Panonychus ulmi, 355
of plum curculio, 351
plant residual toxicity, 332, 333
residual toxicity tests for, 328
Erbkoordination, 212
Ergates, 100, 128
Ergates spiculatus, 121
Eriococcus orariensis, 259 268
Eriosoma lanigerum (woolly apple aphid)
apple rootstocks resistant to, 350
increase due to DDT, 346

insecticide control of, 350
pigment of, 62
population control, 144
Eristalis, 10
Erotylus sexfasciatus, 125
Erythroneura, 90
Erythroneura variabilis (grape leafhopper), 327
Essostrutha fimbriolata, 123
Estigmena chinensis, 125
Estigmenida variabilis, 125
Ethion, 348, 373
Ethological studies of insect behavior, 207-34
afferent mechanisms, 219-24
causation, 211-26
efferent systems, 211-19
cleaning behavior, 226
cocoon construction, 215
color preference, 220
comparative studies and, 226-29
courtship, 214-15
cyclic changes, 215-16
evolution of, 226-29
flower features, 221
genetics of, 228-29
hormone influence, 216
induced spinning, 215
instinctive activities, 212
nest building, 213
procryptic coloration, 208
protective displays, 208
reproductive behavior, 215
social releasers, 224-26
stereotyped motor patterns, 212
Euaresta aequalis, 271
Eucosoma griseana, 278
Eumenis, 222
Eumenis semele, 220
Eumysia, 260
Eupatorium adenophorum control by insects, 252, 253, 259, 261, 268
Euproctis chrysorrhoea, 43, 282, 288
Euproctis terminalis, 168
European cabbageworm, see Pieris brassicae
European corn borer, see Pyrausta nubilalis
European pine sawfly, see Neodiprion sertifer
European spruce sawfly, see Diprion hercyniae
Eurygaster integriceps, 281
Eurynassa australis, 129
Eurytoma, 267
Eusleis, 80
Euscorpius, 209
Eusimulium, 93
Eustromula validum, 118
Euthystira brachyptera, 215
Eutreta xanthochaeta, 261
Euxanthellus, 169
Evaporation, 186
Evolution
and aphids, 139, 140
of aphis host relations, 152-57
arthropod-pathogen combination, 389, 390
of behavior, 226-29
Cerambycidae, 99, 100
of fleas, 392
flower constancy and, 225
host specificity, 105, 106, 107
mimics and, 125
and parthenogenesis, 87
periods of, 99, 100
specialization, 255
Exochomus flavipes, 264

F

Fall webworm, see Hyphantria cunea
Fat body, 1
Fenkapton, 355
Filariasis, 405
Fish
for bioassay of insecticide residue, 337-39
Flea beetles, 374, 378
Fleas
and anemia, 406
and dermatitis, 406-7
and disease, 389-414
evolution of, 392
and filariasis, 405
and murine typhus, 390, 399-403
and myxomatosis, 403
parasites of, 408
and Pasteurella, 390
phylogeny, 392
and plague, 393-97
predators of, 408
and Salmonellosis, 399
taxonomy of, 391-92
Trypanosoma lewisi, 390
and trypanosomiasis, 403-5
and tularemia, 397-99
Tunga infestations, 407-8
Fly, 19
Food habits, 102-5
Fordinae, 142, 146, 155
Forficula, 6, 9
Forficula auricularia, 4
Formica rufa, 46
Formica rufa pratensis, 49
Frankliniella fusca, 379
Frankliniella tritici, 379
Fumea crassiorella, 218
Fungi, 129
high humidity and, 291
and insecticides, 293
pest control with, 280-82
Fungicides
combined with insecticides, 363, 369-74, 376, 380-81
and microbial control, 292-93
systemic, 381
Fusarium, 281

G

Galerucella, 14
Galleria, 9, 10, 215
Galleria mellonella, 2, 216
Gambusia affinis, 337
Gametogenesis, 18, 20
Gammarus pulex, 338
Gammasan, 374
Gastrophysa cyanea, 259
Gaurotes, 100
Generation time, 145
Genetics, 228, 322
Genetta dongolana, 391
Gerania bosci, 123
Gerbillus robustus, 391
Gibberella fujikuroi, 380
Gibberellic acid, 380
Giganteopalpus helleri, 122
Glenea, 99
Glenea spilota, 112, 113
Glossina, 390
Glossina morsitans (Tsetse fly), 196
Glossina palpalis, 323
Glossina tachinoides, 324
Glucose, 26
Gnorimoschema operculella, 166
Goes, 103
Gonioctena, 93
Gorse, 269
Gorytes campestris, 44
Gorytes mystaceus, 225
Gracilia minuta, 104
Grape leafhopper, see Erythroneura variabilis
Graphognathus fecundus, 87
Graphognathus imitator, 87
Grapholitha molesta (oriental fruit moth), 201, 328, 329
Grasshopper
reproductive behavior, 215
tissue culture, 18, 19, 20
embryo, 23
ionic composition, 25, 26
media for, 29
organic acids in, 27
tissue extracts, 28
tracheation in, 22
see also specific names
Great Basin tent caterpillar, see Malacosoma fragilis
Great Basin wireworm, see Ctenicera pruinina noxia
Greenideinae, 146

Gregarina ctenocephalus, 409
Growth-promoting materials, 380-81
Gryllidae, 89
Gryllus
  courtship, 209, 214-15
  instinctive activities, 212
Gryllus bimaculatus, 228
Guthion
  control by
    deciduous fruit insects, 348, 350, 355
    of seed-corn maggot, 373
Gymnaetron antirrhini, 260
Gymnospermae, 105
Gypsy moth, see Porthetria dispar

H

Habrobracon brevicornis, 165
Habrolepis roux, 170, 171
Haematobia stimulans, 192
Haemocytes
  cellular development, 1
  circulating, 2-3
  count of, 3-4
  fixed, 2-3
  functions of
    blood coagulation, 9-10
    connective tissue formation, 10-11
    growth stimulation, 13-14
    immunity, 6-8
    intermediary metabolism, 11-14
    nutritive, 11-12
    oenocytoids, 14
    phagocytosis, 5, 6-7, 10
    phenol metabolism, 13
    protection from metazoan parasites, 7-8
    in protein synthesis, 11
  staining of, 10
  types of, 4-6
Haemonia, 1
Haemopoietic organs, 2-3
Halogeton glomeratus, 272
Haltica pagana, 259, 270
Haltica virescens, 270
Hamamelistes betulae, 64
Hamamelistes spinosus, 64
Hammoderus, 103
Haplosporidia, 282
Harlequin bug, see Murgantia histrionica
Harrisina brillians, 280
Heat, 192-93
  see also Bioclimatics
Heliconius, 222, 224
Heliconius erato hydara, 220
Heliothis zea (corn earworm), 193
Heliotropium europaeum, 272
Helix aspersa, 32
Hellula undalis, 280
Hemiberlesia lataniae, 166
Hemimetabola, 97
Hemiptera
  blood-sucking, 390
  haemocytes, 1, 5, 9
  Müllerian mimicry, 126
  as parasites, 128
  pigments of, 65
  sex attractants, 45
  and Trypanosoma spread, 404
Hemirhipis, 128
Hemisarcoptes malus, 238
Hemocytes, 23, 33
  see also Haemocyte
Hemolymph
  amino acid content, 26
  analyses of, 24
  as culture medium, 22, 25
  organic acids of, 27
  as tissue culture medium, 17
  as medium for, 29
Heptachlor
  control
    of carrot rust fly, 374
    of onion maggot, 374
    of plum curculio, 351
    of seed-corn maggot, 373
    of wireworms, 369-72
  fish sensitivity to, 337-38
  residual toxicity tests, 327, 332
  seed treatment, 366, 378
  T_{50} test and, 336
Herpetomonas ctenocephali, 408
Herpetomonas ctenopsyllae, 409
Herpetomonas debreuli, 409
Herpetomonas pattoni, 408, 409
Heterocampa manteo, 2
Heteropalpus pretiosus, 122
Heteroptera, 45, 142
Heterospilus prosopidis, 243
Hibernation, 120
Hippopsis, 122
Hippopsis lemniscata, 111
Hirsutella, 281, 292
Holometabola, 8, 139
Homaesthesis, 103
Homoptera, 90, 140, 142
Honey bee, see Apis mellifera
Hoplocampa testudinea (European apple sawfly), 351-52
Hoplocerambyx, 117
Hoplocerambyx spinicornis, 108
  emergence of, 113, 114
  longevity of, 113, 115
  mating of, 119
  olfactory sense of, 118
Hoplopsyllus, 403
Hoplopsyllus glacialis, 398
Hoplopsyllus glacialis glacialis, 407
Hormaphidinae, 155
Hormaphis, 155
Hormones, 27, 216
Horogenes molestae, 166, 329
Host
  distribution of parasite progeny in, 240-42
  parasite populations, 243-45
  parasite populations, experimental, 235, 250
  specificity
    attraction to plant, 117
    and biological control, 254
    Cerambycidae, 105-8
    chalcidoids, 94
    in Homoptera, 90
    in Hymenoptera, 166-72
Host-parasite specificity, 389
Host-selection, 106-7
Humidity
  effect on
    Cerambycidae, 130
    host-parasite populations, 238, 239
    insecticide-residues, 324
  and insect development, 185-86
  see also Bioclimatics
Hyalophora cecropia (cecropia moth)
  spermatogenesis, 30
  tissue culture, 18, 22, 23
Hyalopterus pruni, 151
Hybolasius, 102
Hydrogen ion concentration, 31
Hylemya antiqua (onion maggot), 373-74, 380
Hylemya brassicae (cabbage maggot), 374
Hylemya cilicrura (seed-corn maggot)
  control of, 366, 368
  seed treatment for control of, 372-73
  systemic materials in control of, 380
Hylemya floralis (turnip maggot), 374
Hylobius pales, 90
Hylobius radicis, 90
Hylotrupes
  feeding, 115
  mating, 117
  olfactory sense of, 118
  oviposition of, 119
Hylotrupes bajulus
  food of, 104
  length of life, 114
  pest for dry wood, 109

physical factors effect on, 130
Hymenolepis diminuta, 406
Hymenoptera
bacterial control of, 280
biology of parasitic, 161-82
diapause, 163-66
host specificity, 166-72
nutrition, 177-79
respiration, 179
sex determination, 172-77
venom, 179-80
color preference in, 224
embryonic determination, 23
flower feature attractions, 221
marking scents of, 45-46
marking substances, 48
mimics of, 125
orientation in, 224
as parasites, 127, 128
parthenogenesis in, 172-77
sex attractants, 44
taxonomy of, 94
Hypena jussalis, 261
Hypera brunneipennis, 188
Hypera postica, 187, 195, 198
Hypericum androsaemum, 266
Hypericum perforatum (Klamath weed), 259, 265-67
Hyphantria cunea
bacterial control of, 280
microsporidian infection of, 294
protozoan control, 282
virus epizootics in, 292
Hypocephalus, 126
Hypocephalus armatus, 127
Hypolimnus misippus, 220
Hyposoter disparis, 167
Hystrichopsylla, 392
Hystrichopsylla talpae, 409

I

Idechthis canescens
host habitat, 167, 169
host-parasite equilibrium, 243
parthenogenesis in, 172
temperature and humidity influence, 238
Imported cabbageworm, see Pieris rapae
Insect control by seed treatment, 363-88
factors influencing, 363-69
biological differences, 368
chemicals used, 364-66
insect-dependent factors, 368
insect-independent factors, 364-68
microflora, 367-68
planting practice, 367
populations, 368
seed factors, 364
soil, 366
species, 368
insects controlled, 369-78
Insecticides
biological assay for residues
cage method, 328-29
coated cage method, 326-28
coated plate method, 324-26
with Crustacea, 337-39
with fish, 337-39
impregnated cloth method, 323-24
impregnated paper method, 321-23
photomigration method, 334-37
in plant materials, 330-37
on substrata, 320-30
combined with fungicides, 363, 369, 369-74, 376, 380-81
compatability with microbial control, 292-93
deposit, definition, 319
mode of action of, 303-18
aldrin, 307-10
DDT, 303-7
organophosphorus, 310-14
SH-enzyme inhibitors, 314-15
residues
on animal materials, 330-37
in soil, 329
systemic, 378-80
systemic action, 378-80
Insectoverdins, 68-69
Insects and epidemiology of malaria, 415-34
see also Malaria, epidemiology of
Instinctive activities, 212, 213
Instincts, see Instinctive activities
Iphiaulax, 127
Ipochus, 120
Ips, 90
Isaria, 129
Ischnolea, 122
Isodrin, 307
Isoptera, 210
Ites, 125
Ixais castelnaudi, 125

J

Jack-pine budworm, see Choristoneura pinus
Jack-pine sawfly, see Neodiprion americanus banksianae
Jamwonus, 121
Japanese beetle, see Popillia japonica
June beetle, see Phyllophaga lancelata
Juniperus bermudiana, 260

K

Kalotermes flavicollis, 49-50
Kelthane, 355
Kermesococcus ilicis, 61
Khapra beetle, see Trogoderma granarium
Klamath weed
control of by insects, 257, 265-67
see also St. Johnswort and Hypericum perforatum
Korlan, 373
Koster's curse, see Clidemia hirta
Kotochalia junodi (wattle bagworm), 279, 287, 290

L

Lablab podborer, see Adisura atkinsoni
Laccifer lacca, 61
Lachnidae, 155
Lachnosterna, 378
Laemophoeus, 224
Lagocheirus, 112
Lagocheirus funestus, 263, 264, 265
Lamiinae
Batesian mimicry, 125
coloration, 126
cryptic coloration, 122
distribution, 100, 102
economic importance, 110, 111
feeding habits, 115
hibernation, 120
host specificity, 105
as human food, 129
mating, 116, 117
oviposition of, 119
sexual dimorphism, 122
sound production, 118
wingless forms, 126
Lantana camara
biological control of, 258, 260-62
value of, 253
Laphygma exigua, 379
Larch bud moth, see Eucosoma griseana
Larch sawfly, see Pristiphora erichsonii
Lasioceris, 119

Lasioderma serricorne, 200
Lasius alienus, 48-49, 86
Lead arsenate
  control by
    of plum curculio, 350
    of wireworm, 371
  residues of, 319
  soil residual toxicity of, 329
  as stomach poison for codling moth, 345
Leafminers, 379
Lebistes reticulatus (guppies), 337
Legerella grassi, 409
Legerella parva, 408
Lema, 92
Lepidoptera
  bacterial control of, 280
  carotenoids, 60, 61
  color preference, 224
  courtship of, 209
  eyespots, 224
  haemocyte
    count, 5
    nutritive function, 12
  haemopoietic organs of, 2
  parasites of, 8
  physical properties and a definite response, 219
  pigments of, 65, 66, 68
  resistant to Beauveria bassiana, 286
  sex attractants, 40-43
  taxonomy of, 94
  tissue culture, 24
Lepidosaphes beckii, 281
Lepidosaphes newsteadi, 260
Lepidosaphes ulmi (oyster-shell scale), 237, 353
Lepisma saccharina, 209
Leptinotarsa decemlineata (Colorado potato beetle)
  carotenoids of, 60
  comparison of climates for, 199
  fungi control of, 281
  haemocyte count, 3, 4, 6
  potential infestability of Germany by, 197
Leptocorisa, 125
Leptohylemyia coarctata, 374
Leptomonas pulicis, 409
Leptopsylla segnis, 409
Leptopsyllus musculi, 401
Leptospermum scoparium, 259, 268
Leptostylus, 111
Leptostylus terraecolor, 111
Leptothorax tuberum unifasciatus, 49
Leptura, 104, 112
Leptura ruba, 104
Lepturges spermophagus, 111
Lepturinae
  Batesian mimicry, 124, 125
  distribution of, 100, 102
  feeding habits, 115
  host specificity, 105
  mating, 117
  oviposition of, 119
  sound production, 118
  symbiotes of, 103
  wingless forms, 126
Lepturini, 116, 119, 123
Lepus americanus, 398
Lethocerus indicus, 45
Leucographis, 119
Light, 345, 353
  see also Bioclimatics
Light intensity, 187
Lime-sulphur, 328
Liminites, 220, 224
Limonius agonus (eastern field wireworm), 372
Limonius californicus (sugar-beet wireworm), 368
Limonius canus (Pacific Coast wireworm), 366, 368
Linaria dalmatica, 272
Linaria vulgaris, 260, 272
Lindane
  control by
    of carrot rust fly, 374
    of flea beetles, 378
    of seed-corn maggot, 372-73
    of wheat bulb fly, 374
    of wireworms, 364, 365, 368, 369-72
    of woolly apple aphid, 350
  fish sensitivity to, 337
  masking technique, 323
  overdosage of, 376
  plant residual toxicity testing for, 332, 333
  residual toxicity of, 324, 326-28
  seed treatment, 366-68, 378
  spray treater for applying, 377
  tests for residue, 324
  T_{50} test and, 336
  see also γ-BHC
Liothrips urichi, 267
Liponyssus bacoti, see Ornithonyssus bacoti
Liriomyza, 179, 379
Lissorhoptrus orysophilus (rice water beetle), 378
Lithocolletis, 349
Lithocolletis crataegella, 349
Lithophane antennata, 353
Lixus algirus, 270
Locusta migratoria, 3, 61
Locusta migratoria migratoriodes, 284
Loemopsylla cheopsis, 391
Longitarsus, 374, 378
Lophogogonius crinitus, 112
Lophopoeum timbouvae, 111
Loxostege, 164
Loxostege frustralis, 169
Loxostege sticticalis, 169
Lucilia cuprina, 28
Lucilia sericata, 311
Lycaena phlaeas, 78
Lygaeidae, 65
Lygesis, 125
Lygidea mendax, 353
Lygus, 327
Lymantria dispar, see Porthetria dispar
Lymantria monacha (Nun moth), 284, 292
Lyonetia clerckella, 353

M

Macrocentrus ancylivorus, 322, 329
  destruction by Nosema destructor, 294
  oviposition rate and humidity, 185
  rearing of, 166
  sex ratio of, 176
Macrodontia, 121
Macropis labiata, 44
Macrosiphoniella sanborni, 321
Macrosiphum pisi, 285, 380
  see also Acrythosiphon pisum
Macrosiphum solanifolii, 281
Macrotoma, 121
Macrotoma hayesi, 122
Macrotoma palmata, 104
Magicicada septendecim, 353
Malacosoma, 18
Malacosoma disstria, 278
Malacosoma fragilis, 278, 290
Malacosoma neustria, 282
Malaria
  endemic, 419
  epidemiology of, 415-34
    Anopheles dispersal by transportation, 420
    basic principles, 415-16
    mathematical, 416-20
    Plasmodium life cycle, 415, 417
    reproduction rate, 418
    vector density, 418
  eradication, 421, 430, 431
  status of, 420-31
  vectors of, 420, 421, 422-25
    species lists, 420-31
Malathion, 314, 350
Mallambyx raddei, 116
Malleomyces pseudomallei,

409
Mallophaga, 390
Malpigiella refringens, 409
Manuha, 268
Margasus afziella, 128
Marmota bobac, 396
Marmota monax, 402
Massospora, 285
Mating, 116
Mealworm, see Tenebrio molitor
Mecas, 103
Mecas saturnina, 271
Meconema varia, 61
Media for tissue culture, 24-30
Mediterranean flour moth, see Anagasta kühniella
Mediterranean fruit fly, see Ceratitis capitata
Megabothris walkeri, 398
Megachile, 125
Megacyllene, 128
Megacyllene antennatus, 129
Megacyllene caryae, 129
Megacyllene robiniae, 112
Megasoma elephas, 221
Megopis, 108
Megoura viciae, 108, 147, 148
Melanargia galathea, 65
Melanins, 73
Melanoplus mexicanus, 78
Melanoplus puer, 84, 89
Melanoplus spretus, 78
Melanotus, 372
Melaphis, 142
Melastoma malabathricum, 272
Melipona, 125
Melitara dentata, 259
Melitara doddalis, 265
Melitara prodenialis, 265
Melittobia, 175
Melolontha, 279, 294
Melolontha hippocastani, 329
Melolontha melolontha, 279, 291
Meltzerella lutzi, 125
Mercuric chloride, 271
Mermiria maculipennis, 89
Meroscelisus, 127
Meroscelisus violaceus, 123
Mesoleius tenthredinidis, 7, 170
Mesosini, 124
Metabolism
  haemocytes in intermediary, 11-14
  oxidative, effect of DDT, 330-5
  phenol, 13
  and tissue culture, 33
Metamorphosis
  glycogen and, 11
  and hormones, 27
  phagocytosis, 6
  vitamin deficiency, 53
Metaphycus helvolus, 165, 178
Metarrhizium anisopliae, 281, 287
Metatetranychus citri, 281
Methia, 112, 125
Methiini, 125
Methoxychlor
  control by
    of codling moth, 347
    of plum curculio, 351
  effect on oxidative metabolism, 303
  fish sensitivity to, 337-38
  plant residual toxicity testing for, 332
  residual toxicity tests for, 328
  soil residual toxicity of, 329
  T_{50} test and, 336
Mexican bean beetle, see Epilachna varivestis
Mexican fruit fly, see Anastrepha ludens
Microbial control of pests, 277-302
  compatibility
    with adjuvants, 292-93
    with antibiotics, 292-93
    with fungicides, 292-93
    with insecticides, 292-93
    with other biological control agents, 293-94
  effect of physical environment, 291-92
  host resistance, 286-87
  infection, 283-84
  methods of application, 288-90
  methods of dispersal, 284-86
  persistence in field, 290-91
  transmission of, 283-84
  use of pathogens, 278-83
    by algae, 282-83
    by bacteria, 279-80
    by fungi, 280-82
    by nematodes, 282-83
    by protozoa, 282
    by viruses, 278-79
  virulence, 287-88
Microctonus gastrophysae, 259
Microctonus vittatae, 169
Microcyltus, 103
Micropera obietum, 109
Microsporidia, 282, 293
Microtus pennsylvanicus pennsylvanicus, 402
Migration, 148-50
Migratory grasshopper, see Melanoplus mexicanus
Milky-disease, see Bacillus popilliae
Mites, 281, 346
  spider, 379
Mitosis, 18-29, 33
Moisture
  in climate classification, 189, 190
  effect on host-seeking behavior, 219
  limiting insect distribution, 195-97
  seed treatment, 364
  see also Humidity
Molorchus, 112, 125
Moneilema, 126, 259, 265
Moneilema armata, 265
Moneilema crassa, 265
Moneilema ulkei, 112, 263, 264
Moneilema variolare, 263
Monochamus, 115
  digestive enzymes, 105
  economic importance, 109
  feeding habits, 115
  mating, 117
Monochamus scutellatus, 129
Monochamus titillator, 130
Monopsyllus sciurorum, 409
Morimopsini, 126
Morimus, 126
Mormoniella, see Nasonia
Mormoniella vitripennis
  host finding, 167, 219, 245
  host protection against, 244
Morpho, 123
Morphogenesis, 19, 20, 33
Mortality, 127-30, 241
Mosquitoes
  malarial parasite of, 24
  sexual isolation, 94
  tissue culture, 19, 20, 22, 25, 26
    media for, 28, 29
Motor patterns, 219
Murgantia histrionica (Harlequin bug), 193
Murine typhus, 390
Musca
  haemocytes of, 2
  haemopoietic organ, 3
  instinctive activities, 212
  tissue culture, 18
Musca domestica
  ACH in, 306, 312
  aldrin effect on, 307, 309
  γ-BHC effect on, 307
  cleaning behavior, 226
  DDT effect on, 304, 306
  DDT residue testing with, 321, 325-27
  DDVP effect on, 314
  DEP effect on, 312
  determining insecticides in milk with, 333, 334
  DFP effect on, 313
  insecticidal residues in, 330-31
  lindane residue and, 325
  malathion effect on, 314
  parasite for, 245

sexual pump release, 223
SH-enzyme inhibitors, 314-15
tests for insecticide residue, 321
Musca domestica vicina, 326
Mus gentilis, 391
Mus musculus, 402
Mycobacterium leprae, 409
Myiophagus, 281, 292
Myrica faya, 272
Myrmecophily, 155-56
Myrmeleon, 167
Myxomatosis, 403
Myzus persicae
color contrast in, 150
feeding of, 140, 141
host selection, 151, 152
migration of, 149, 150

N

Naedius elegans, 125
Nasionia, 164
Nasonia vitripennis, 167
Nassanoff pheromone, 46
Necydalini, 124
Nematocera, 226
Nematodes
high humidity and, 291
pest control with, 282-83
physical factors and, 291
Nematus vagus, 94
Nemeritis canescens, 8, 167
Nemobius, 89
Nemobius carolinus, 228
Nemobius confusus, 228
Nemobius maculatus, 228
Nemobius melodius, 228
Neoaplectana glaseri, 288
Neoaplectana leucaniae, 285, 288
Neocatolaccus mamezophagus, 243, 245
Neoclytarlus, 108
Neoclytus, 107, 128
Neoclytus conjunctus, 112
Neodiprion, 94
Neodiprion americanus banksianae, 278
Neodiprion sertifer, 278, 290, 292
Neonema ctenophthalmi, 405
Neonitocris, 125
Neonitocris princeps, 128
Neopsylla setosa, 398
Nezara viridula, 65
Nicotine, 351
Nomenclature, see Classification and Taxonomy
Noogoora bur, 271
Nosema, 19
Nosema bombycis, 282
Nosema ctenocephali, 408
Nosema destructor, 294
Nosema infesta, 282
Nosema lymantriae, 288
Nosema pulicis, 408
Nosopsyllus fasciatus, 399, 401, 406, 408, 409
Nothorhina, 119
Notonecta, 219
Nun moth, see Lymantria monacha
Nupserha, 120
Nupserha antennata, 260, 271
Nutgrass, 271-72
Nutrition
of aphids, 143-44
cellular, and tissue culture, 33
Hymenoptera, parasitic, 177
Nyctemera annulata, 269
Nygmia phaeorrhoea, 282

O

Oak processionary caterpillar, see Thaumetopoea processionea
Oberea, 102
damage by, 110
oviposition of, 119, 120
Oberea bimaculata, 110
Obriini, 122
Ochraethes, 123
Octotoma plicatula, 261
Octotoma scabripennis, 261
Odonata
courtship of, 209
embryonic determination, 23
sexual pursuit flight, 221
tissue element, 10
Odontopsyllus, 403
Odontopsyllus multispinosus, 398
Oecanthus, 89
Oedipoda, 227
Oedipoda caerulescens, 61
Oedipoda miniata, 61
Oikocytes, 1
Olfactory sense, 118
Olycella junctolineela, 263
Ommochromes, 69-73
Oncideres, 102, 119, 120
Oncideres cingulatus, 120
Oncideres dejeanii, 69, 120
Oncopeltus, 11
Onychiurus, 373, 378
Operophtera brumata, 349
Ophiomyia lantanae, see Agromyza lantanae
Opisocrostis, 408
Opius tryoni, 252
Opuntia, 252, 253
Opuntia aurantiaca, 263, 264
Opuntia dillenii, 263
Opuntia elatior, 263
Opuntia megacantha, 262-65
Opuntia tuna, 265
Opuntia vulgaris, 262-65
Orchopeas sexdentatus, 404
Orchopeas wickhami, 404, 408
Oriental fruit moth, see Grapholitha molesta
Ornithodoros, 389
Ornithonyssus bacoti, 401
Orteguaze, 122
Orthezia annae, 260
Orthopodomyia, 94
Orthoptera
carotenoids of, 60, 61
courtship, 209
embryonic determination, 23
nutrition of, 143
ommochromes, 69
pigments of, 65, 68
sex attractants, 44
songs of, 221
taxonomy of, 89-90
Oryctes, 2, 18
Oryctes nasicornis, 1
Oryctes rhinoceros, 281
Oscinella frit (frit fly), 374
Ostrya virginiana, 92
Otiorhynchus ligustici, see Brachyrhinus ligustici
Otiorhynchus scaber, 87
Overwintering, see Hibernation
Ovex, 328
Ovigenesis, 178
Oviposition, 119-20
Oxymirus cursor, 104
Oystershell scale, see Lepidosaphes ulmi

P

Pachyrrhynchus, 125
Pachyteria, 117
Pacific Coast wireworm, see Limonius canus
Pale western cutworm, see Agrotis orthogonia
PAM, 313, 314
Pamakani, 268
Panonychus ulmi (European red mite), 346, 353-55, 356
Paramecium bursaria, 242
Paramecium caudatum, 242
Parandra, 108
Parandrinae
distribution, 100, 102
host specificity, 105
oviposition of, 119
Paraprocipihilus, 155
Parasites
aphids as, 140-46
of Cerambycidae, 127-29
definition of, 161-62
density relationships of host and, 241
distribution of among hosts, 240
effect on populations, 236

host populations, 243-45
metaxoan, 7,8
Parasitoid, 161
Parathion
control by
of apple flea weevil, 351
of codling moth, 347, 348
of European apple sawfly, 352
of flea beetles, 378
of Panonychus ulmi, 355
of plum curculio, 351
seed treatment, 278
of woolly apple aphid, 350
lethal action of, 313
and microbial control, 293
residual toxicity testing in, 324, 327, 329, 331-33
T_{50} test and, 336
Paratimia conicola, 107
Paris Green, 371
Parlatoria oleae, 166
Parmenini, 124, 126
Parthenogenesis
in aphids, 140
evolution, 87
in Hymenoptera categories of, 172-73
taxonomy in, 87-88
Pasteurella pestis, 390, 393-97
Pasteurella tularensis, 390, 397-99
Pauridia peregrina, 171
Pea aphid, see Acyrthosiphon pisum and Macrosiphum pisi
Pectinophora gossypiella (pink bollworm), 197
Pediculus humanus, 2, 39, 323
Pegohylemyia jacobaeae, 270
Pemphigidae, 142, 147, 154
Penicillium frequentans, 368
Penicillium gladioli, 368
Pentatomidae, 65
Peptone, 27
Perezia pyraustae, 282
Peridroma margaritosa, 32
Perilitus coccinellae, 169
Periphyllus, 146, 153
Periplaneta americana (American cockroach)
ACH effect on, 306, 311
aldrin effect on, 309
γ-BHC effect on, 305, 309
DDT effect on, 304, 305, 306, 307, 309
haemocytes of, 2
pigments of, 68
sex attractants, 44
SH-enzyme inhibitors in, 315
TEPP effect on, 312
tissue culture, 28, 29
Periplaneta fuliginosa, 305
Perisierola emigrata, 179
Peromyscus leocupus noveborascensis, 402
Perperus vermiculatus, 351
Petrognatha, 122
Petrognatha gigas, 119
Phacellus, 123
Phaedon cochleariae, 380
Phagocytosis, 5, 6-7, 10-11
Phanerotoma grapholithae, 329
Phaonia, 3
Pharsalia saperdoides, 122
Pheidole pallidula, 48-49
Pheromones, 39-58
definition, 39-40
olfactory acting, 40-46
marking scents, 45
sex, 40-45
orally acting, 47-53
ant queen, 48-49
honey bee queen, 47-48
royal jelly determining factor, 51-53
of termites, 49-51
Philanthus, 224
Philini, 126
Philosamia ricini, 61
Philus, 111
Phlebotomus, 405
Phoracanthini, 122
Phormia, 2
Phormia regina, 218
Phratora purpurea, 92
Phryganidia californica (California oakworm), 292
Phryneta, 119
Phryneta aurocincta, 119
Phryneta spinator, 129
Phyllophaga lancelota, 44
Phyllotreta, 169, 374, 378
Phylloxeridae, 63, 140, 142
Phylogeny, 227, 392
Phymata, 128
Phymatodes, 104
Physalis, 92
Physcus, 169
Physocnemum brevilineum, 103, 105
Physonota alutacea, 267
Phytoecia, 111, 119
Pidonia, 103
Pieridae, 66, 68, 282
Pieris, 2, 222
Pieris brassicae
Apanteles glomeratus infection in, 294
carotinoids of, 60, 61
color preference, 220
control by microbes, 279, 280
food ingestion rate, 141
limits on distribution, 194
parasite relationship, 164
persistence of bacterial infection in, 290
pigments of, 68
Pieris bryoniae, 219, 220
Pieris napi, 219, 220
Pieris rapae
carotenoids, 60
control by microbes, 279, 280
virus infections in, 287
Pigments, 59-76
anthocyanins, 65
anthoxanthins, 65
anthraquinones, 61-62
aphrins, 62-64
bile, 68-69
carotenoids, 60-61
insectoverdins, 68-69
melanins, 73
ommochromes, 69-73
pterins, 65-68
see also Coloration
Pimpla bicolor, 168
Pinaceae, 106
Pine processionary caterpillar, see Thaumetopoea pityocampa
Pink bollworm, see Pectinophora gossypiella
Pipturus mysus, 108
Piri-piri, 270-71
Pissodes, 90
Plagiohammus spinipennis, 112
Plagithmysus, 119
host specificity, 108
Plagodis, 80
Plague, 391, 393-97
bacillus
first discovery, 393-94
and fleas, 393-97
history of, 393-97
sylvatic, 391
Xenopsylla cheopis as carrier, 395-97
Plasmodium falciparum, 415, 417
Plasmodium malariae, 415, 417
Plasmodium ovale, 415, 417
Plasmodium vivax, 415, 417
Platycnemis, 221
Platyptilia pusillidactyla, 261
Platysamia cecropia, 215
cocoon construction, 215
Plectrodera, 119
Plectrura, 100, 120
Plesiocorus rugicollis, 353
Plodia, 43
sex attractants, 43
Plum curculio, see Conotrachelus nenuphar
Plusia gamma, 279
Plutella maculipennis

DDT relations with, 321
host finding of parasite for, 168
microbial control of, 280
mortality in, 237
Podisma, 227
Poekilosoma, 122
Pogonocherus hispidus, 120
Polistes, 210, 214
Polistes exclamans, 294
Polychrois botrana, 43
Polyembryony, 177
Polyethylene glycol, 366
Pompilidae, 227
Pompilus plumbeus, 228
Popillia, 9, 10
Popillia japonica (Japanese beetle)
control by rickettsiae, 279
insecticide residual toxicity tests for, 321, 327
milky-disease, 290
moisture and distribution, 196
nematode infection in, 288
Population
density-dependent mortality factor, 295
dynamics, 161, 251
abiotic, 251, 252
biotic, 251, 252
experimental host-parasite, 235-50
biotic interactions effect, 240-47
relation to natural, 236-37
in fields of aphids, 144-46
host-parasite, 243
physical environment effect on, 237-39
models of host-parasite interaction, 245
mortality of, 127-30
insect disease as factor, 294-95
natural, 237
parasite and host, 168
predator-prey, 242-43
regulation of by diseases and parasites, 293
and seed treatment, 368
Populus, 91, 92
Populus balsamifera, 91, 92
Porthetria dispar
microsporidian infection in, 288
sex attractant, 42-43
stresses and virus infections, 292
Potato aphid, see Macrosiphum solanifolii
Potato flea beetle, see Epitrix cucumeris
Potato tuberworm, see Gnorimoschema operculella
Prairie grain wireworm, see Ctenicera aeripennis destructor
Predators
of Cerambycidae, 127-29
as distinguished from parasite, 161
prey populations, 242-43
see also Parasite
Prickly pear, 262-65
see also Opuntia
Priocnemis exaltatus, 228
Prionapterus, 122, 127
Prioninae
coloration of, 123
cryptic, 122
distribution, 100, 102
economic importance, 111
flight of, 116
host specificity, 105
as human food, 129
longevity of, 113
mating, 117
oviposition of, 119
sexual dimorphism, 121
wingless forms, 126
Prionocalus, 126
Prionoma, 122
Prionus, 103, 112, 121, 128
Prionus californicus, 121
Pristiphora erichsonii (larch sawfly), 7, 170, 280
Pristiphora quercus, 94
Probatius choliniformis, 125
Procecidochares utilis, 252, 268
Prociphilini, 154
Prodenia, 11, 12
Prodenia eridania, 4
Prodenia litura, 43
Promethea moth, see Callosamia promethea
Prosoplus banki, 115
Prospaltella perniciosi, 173
Protein synthesis, 11
Protoparce quinquemaculata, 280
Protoparce sexta, 280
Protazoa, 282
Prunus americana, 92
Psalidocoptus, 126
Psalidognathus, 121, 123
Psalidognathus superbus, 121
Psallus seriatus (cotton leafhopper), 379
Psenocerus, 120
Pseudaletia unipuncta, 288
Pseudemys, 95
Pseudomonas aeruginosa, 280, 284, 289
Psila rosae (carrot rust fly), 374
Psylliodes chrysocephlala, 378
Pterins, 65-68
Pterocomma smithiae, 65
Pteroplatini, 124
Ptinus tectus, 200
Pulex cheopis, 391
Pulex irritans, 399, 405, 406
Pulex murinus, 391
Pulex philippinensis, 391
Pulex tripolitanus, 391
Pulvinaria, 13
Pulvinaria mesembryanthemi, 5
Pupipara, 226
Purpuricenus, 112
Pyrausta, 164, 165
Pyrausta nubilalis
Bacillus thuringiensis as control for, 280, 283
comparison of climates for, 199
microsporidia in, 283, 286
Pyrethrin, 310, 322, 325
Pyrethrum, 319
Pyrgus, 78
Pyrodes, 123, 124
Pyrolan, 322
Pyrotrichus vitticollis, 103
Pyrrhocoris apterus, 60, 65
Pythium ultimum, 367

Q

Q fever, 400

R

Ranova, 119
Rattus, 402
Red-banded leaf roller, see Argyrotaenia velutinana
Reduviids, 9
Reproductive behavior, 215
Rhagionidae, 128
Rhagionycha fulva, 117
Rhagium, 103
digestive enzyme in, 104, 105
emergence of, 112
feeding habits, 115
parasite of, 128
Rhagoletis cingulata (cherry fruit fly), 328
Rhagoletis pomonella (apple maggot), 352-53
Rhinatragini, 125
Rhinoceros beetle, see Oryctes rhinoceros
Rhinocorus nitidulus, 128
Rhipidocerus, 121

Rhizobium leguminosarum, 373
Rhizoctonia, 369
Rhodnius
  blood coagulation in, 9
  haemocytes, 1, 2
    nutritive role of, 11-14
    types of, 4
  metamorphous in, 6
  tissue formation, 10
Rhodnius prolixus, 24, 69
Rhopalocera, 224, 227
Rhopalopus, 100
Rhopalosiphum maidis, 142, 150
Rhyacionia buoliana (European pine shoot moth), 193
Rhynchaenus pallicornis (apple flea weevil), 351
Rhynchites pauxillus, 351
Rhynchophora, 90
Rice water beetle, see Lissorhoptrus oryzophilus
Rice weevil, see Sitophilus oryza
Rickettsia burnetii, 409
Rickettsia rickettsii, 400
Rickettsia typhi, 399-403
Rocky mountain grasshopper, see Melanoplus spretus
Romaleum, 105
Romaleum atomarium, 130
Ropica dorsalis, 111
Rosaceae, 271
Rosalia, 100
Rotenoid, 337
Ryania, 348

S

St. Johnswort (Klamath weed), 258, 265-67
Salicaceae, 154
Salix, 91, 92
Salmonella enteritidis, 399
Salmonella typhimurium, 399
Salmonellosis, 399
Salvia aethiopis, 272
Sembucus, 103
Samia cynthia, 30
San Jose scale, see Aspidiotus perniciosus
Saperda, 103
  damage by, 110
  host specificity, 106, 107
  oviposition of, 119
Saperda calcarata, 118, 127
Saperda carcharias, 129
Saperda hornii, 112
Saperda tridentata, 110
Saphanini, 103
Sarcophaga, 3, 9, 13
Sarcophaga bullata, 5, 13
Sarcophaga caranaria, 226
Sarcophaga falculata, 5
Sarifer flaviramus, 121
Saturnioidea, 228
Sawfly, 2
Scales, 281, 292
Schematiza cordiae, 256, 259, 267
Schistocera gregaria, 141, 143, 218
Schizogregarina, 282
Schizonympha, 378
Schoenobius incertulus, 380
Schradan
  control by
    of cotton insects, 379
    of frit fly, 374
  as systemic agent, 379-80
Schreckstoffe, 40
Schreiteria bruchi, 126
Sciadella saltator, 127
Sciara, 93
Scolytus rugulosus, 353
Scopeuma stercorarium, 192
Screw-worm, see Callitroga hominivorax
Seed treatment for insect control, 363-88
Selection, ethological, 169
Semanotus, 106
Senecia jacobaea, 259, 269-70, 272
Sevin, 348
Sex
  attraction by scent, 220
  determination
    in arrhenotokous species, 174-76
    in parasitic Hymenoptera, 172-77
    meiosis in the haploid male, 174
    viability of haploid males, 173-74
  dimorphism, 125
  forms in aphids, 146, 147
  isolation and songs, 221
  jump in Musca domestica, 223
  ovigenesis, 178
  ratio and
    mechanics of fertilization, 176-77
  responses to scent, 222
Sex attractants, see Pheromones, olfactory acting
SH-enzyme inhibitors, 314-15
Silkworm, see Bombyx mori
Siphona irritans, 192, 334
Siphonaptera, 390
Sitophilus granarius, 191-92
Sitophilus oryza (rice weevil), 78, 191-92, 327
Smerinthus ocellatus, 208
Smodicum, 104, 121
Smynthurus viridis, 195
Social releasers, 224-26
Solenopsis molesta (thief ant), 378
Solenotus begini, 179
Somatidia, 102
Songs, 209, 221
Southern corn rootworm, see Diabrotica undecimpunctata
Spalacopsis, 121, 122
Spalangia drosophilae, 163, 164
Species, see Taxonomy
Spermatogenesis, 174
  metabolic inhibitors of, 30
  tissue culture, 20, 22
Sphingidae, 228
Sphinx ligustri, 61, 68
Spiders
  courtship, 224
  stereotyped motor patterns of, 212, 214
  waning of response, 218
  web building comparison, 227
Spilonota holotrephas, 259
Spilonota ocellana, 353
Spilopsyllus cuniculi, 398, 403, 404
Spirochaeta ctenocephali, 409
Spondylinae
  distribution of, 100
  host specificity, 105
  oviposition, 119
  symbiotes of, 103
Spondylis, 100, 112, 119
Sporotrichum globuliferum, 129
Spotted alfalfa aphid, see Therioaphis maculata
Spotted fever, 400
Spruce budworm, see Choristoneura fumiferana
Stachytarpheta jamaicensis, 272
Stagmomantis limbata, 89
Staphylococcus albus, 7
Starvation tests, 255-56
Steina rotundata, 409
Steinernematidae, 282
Stenagostus, 128
Stenaspini, 122, 123
Stenobracon deesae, 170
Sterilization, 32
Steracanthus undatus, 125
Stereotyped motor patterns, 212
Sternotomini, 123
Stictocephala bubalus, 353
Stomatoceras rubrum, 167
Stomoxys calcitrans, 334
Strepsicrates smithiana, 272

Stromatium, 115, 119
Stromatium barbatum, 106, 109, 113
Stromatium fulvum, 104
Stromatium longicorne, 109
Strychnine, 371
Styloxus, 102, 112
Sugar-beet wireworm, see Limonius californicus
Sybra alternans, 111
Syllitus, 117
Sylvilagus braziliensis, 403
Sylvilagus floridanus, 398
Symbiosis, 103-5, 162
Symbiote, 103, 144
Synaphaeta, 112
Syngamia haemorrhoidalis, 261, 262
Syrphus ribesii, 7
Systematics, see Taxonomy
Systox, 350, 355
  see also Demeton

T

Tabardillo, 399
Tansy ragwort, 269
Tarsonemus pallidus, 242, 243
Taxodiaceae, 106
Taxonomy
  Cerambycidae, 100
  concepts of, 79-88
    multidimensional species, 83-87
    nondimensional species, 81-83
    parthenogenetic forms, 87-88
    polytypic species, 83-87
    subspecies, 85-86
    typological species, 79-81
  of fleas, 391-92
  methods, 88-95
    biochemical, 95
    Coleoptera, 90-93
    Diptera, 93-94
    Homoptera, 90
    Hymenoptera, 94
    Lepidoptera, 94-95
    Orthoptera, 89-90
  species definition, 79
  see also Classification and Nomenclature
TDE, 328, 332
Telemone, 54
Teleonemia lantanae, see Teleonemia scrupulosa
Teleonemia scrupulosa, 261, 262
Teleonemia vanduzeii, 261
Temnochila, 127
Temperature
  in climate classification, 189, 190
effect on
  aphids, 147
  Cerambycidae, 129-30
  emergence, 115
  host-parasite populations, 238, 239
  host-seeking behavior, 219
  insecticidal properties, 327
  insecticide residues, 324
  males in deuterotokous species, 176
  sexuality of uniparental, 172
  treated seed, 366
and humidity, 185-86
insect development, 184-85
limiting insect distribution
  cold, 192-94
  heat, 194-95
in tissue culture, 30
Tenebrio, 3, 4
Tenebrio molitor
  DDT effect on, 304
  haemocytes of, 2
    and parasites, 8
  sex attractant, 44
Tenebroides, 127
Tenthredinidae, 2
TEPP
  effect on roaches, 312-14
  lethal action of, 310, 313
  microbial control and, 293
Tereticus, 121
Termites, 49-51, 227
Tetanops myopaeformis (sugar-beet root maggot), 378
Tetranychus, 346
Tetranychus desertorum, 263
Tetranychus opuntiae, see Tetranychus desertorum
Tetraopes, 103, 123
Tetrastichus, 259
Tetrix, 227
Tetropium, 112
Tetropium gabrieli, 106, 129
Tettigonia, 61
Tettigoniidae, 89
Thanasimus, 127
Thaumetopoea pityocampa, 278, 279
Thaumetopoea processionea, 280
Thecla bazochi, 261, 262
Thecla echion, 255, 261
Thelaxidae, 142, 146, 155
Thelohania hyphantriae, 282
Thelohania similis, 288
Thelyotoky, 172
Therioaphis maculata (spotted alfalfa aphid), 380
  biological and microbial control of, 281, 293
  in distribution of fungi, 285
  feeding habits of, 142
  fungi infection, 291
  seed treatment and, 380
Thimet
  control by
    of cotton insects, 379-80
    of onion maggot, 374
    of seed-corn maggot, 373
  seed treatment, 369, 378
  as systemic agent, 379-80
Thiram, 374, 376
Thonalmus, 126
Thrips, 379
Thrips imaginis, 353
Thrips tabaci, 379
Thyronectia balsamea, 109
Ticks, 320, 389
Tilia americana, 92
Tillomorphini, 103, 124
Tillyardia gigas, 122
Tiphia matura, 167
Tissue culture, 17-38
  amino acids, 26-27
  antibiotics in, 31-32
  characteristics of, 21-30
  culture flasks, 30-31
  embryonic determination, 23
  energy source, 26
  hydrogen ion concentration, 31
  media in, 24-30
    adenosine triphosphate, 27
    amino acids, 26-27
    conditioned, 25
    energy sources, 26
    hormonal environment, 27
    ionic composition, 25
    nucleic acids, 27
    other organic acids, 27
    tissue extracts, 28-29
    vitamins, 27
  methods of sterilization in, 32
  morphogenesis and, 33
  organ, 23-24
  and pathology, 34
  pharmacology, 33-34
  sensitivity to growth inhibitors, 32
  separation of cells, 32
  simple epithelia, 21
  substrates, 31
  temperature in, 30
  tracheation in, 22
  uses of, 33-34
Tissue formation, 10-11

Torymus druparum, 353
Toxaphene
control by
of European apple sawfly, 352
of seed-corn maggot, 373
fish sensitivity to, 337-38
measurement of potency, 322
residual toxicity testing, 327, 330, 332
T_{50} test and, 336
Toxoti, 103
Tracheation, 22
Tragiscoschema bertolonii, 111
Tragiscoschema wahlbergi, 111
Tragoscephalini, 123
Tragsoma, 121
Trialeurodes abutilonea, 379
Trialeurodes vaporariorum, 168, 239
Triatoma, 404
Tribolium, 53
Tribolium castaneum, 248, 321, 323, 325, 328
Tribolium confusum, 248, 322, 325
Tribulus terrestris, 272
Trichodectes canis, 406
Trichoderma viride, 368
Trichogramma cacoeciae, 164
Trichogramma minutum, 271
Trichomesiini, 103
Trichoplusia ni, 279, 293
Trirhabda pilosa, 260
Trithion, 348, 355, 373
Tritogenaphis rudbeckiae, 65
Trogoderma granarium (Khapra beetle), 200
Trophamnion, 177-78
Trypanosoma citelli, 404
Trypanosoma cruzi, 390, 404
Trypanosoma duttoni, 404
Trypanosoma evotomys, 404
Trypanosoma leporis-sylvaticus, 404
Trypanosoma lewisi, 390, 403-5
Trypanosoma nabiasi, 404
Trypanosoma neotomae, 404
Trypanosoma parkeri, 404
Trypanosoma peromysci, 404
Trypanosoma rabinowitschi, 404
Trypanosoma soricis, 404
Trypanosomiasis, 403-5
Tsetse fly, see Glossina morsitans
Tuber flea beetle, see Epitrix tuberis
Tuberolachnus salignus, 63
feeding habits of, 140, 141 142
nutrition of, 143, 145
Tucumania tapiocola, 263
Tularemia, 397-99
Tunga, 392
Tunga penetrans, 407-8
Typhlocyba froggatti, 353
Typhlodromus, 242, 243
Typhlopsyllus assimilis, 404
Typhus
endemic, 399
epidemic, 400
flea-borne, 399, 400
Mexican, 399
murine, 399-403
Tyria jacobaeae, 259, 269-70

U

Ulex europaeus, 254, 269
Ulmus americana, 92
Uracanthus cryptophagus, 110
Uropoda, 408

V

Vanessa, 69
Vanessa urticae, 70
Verticillium, 281
Vespa, 20
Vesperini, 126
Verperus, 103, 111
feeding habits, 115
oviposition of, 119
rootfeeding adaptations, 127
Viruses
in Cerambycidae, 129
diseases, control of, 380
and insecticides, 293
latent infections, 284, 286
persistence of, 290
pest control with, 278-79
physical factors and, 292
Vitamins, 27, 52
Viteus vitifoliae, 142, 143
Volumnia westermanni, 111

W

Wasps, 124, 126
Wattle bagworm, see Kotochalia junodi
Weather, 324
see also Bioclimatics
Web building, 212, 214
Western raspberry fruitworm, see Byturus bakeri
Wheat sawfly, see Cephus cinctus
Wireworms
control of, 364, 365, 368, 369-72
Woolly apple aphid, see Eriosoma lanigerum

X

Xanthium, 260
Xanthium pungens, 271
Xanthium strumarium, 271
Xaurus bennigseni, 121
Xenopsylla cheopis
history of, 391
murine typhus in, 400, 401
as plague carrier, 395-99
Xenopsylla pachyuromydis, 391
Xenopsyllus cleopatrae, 409
Xixuthrus heros, 121
Xixuthrus microcerus, 121
Xordini, 128
Xyloteles, 102
Xylotrechus, 107, 112, 128
Xylotrechus insignis, 103
Xylotrechus quadrimaculatus, 102
Xylotrupes, 11
Xystrocera globosa, 104-15
Xystrocera nyassae, 117

Z

Zelliboria, 125
Zeuxideplosis giardi, 265, 266
Zootermopsis, 50
Zygaena carniola, 285
Zygaenobia intestinalis, 285
Zygrita diva, 123